HOSPITAL LIBRARY

VOLUME 85 NUMBER 4

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

October, 1949

Studies of Respiratory Air Flow. I. Significance of the Normal Pneumotachogram. Donald F. Proctor and Janet B. Hardy 253

Histochemical Studies on Cartilage and Bone. I. The Normal Pattern. 1,2. Richard H. Follis, Jr. and Morgan Berthrong 281

Acute Hepatitis in General Hospital Practice. The Role of Transfusions and Inoculations. Oscar D. Ratnoff and George S. Mirick 299

Colonmetrographic Studies of the Effects of Section of the Parasympathetic Nerves of the Colon. H. William Scott, Jr., and James R. Cantrell 310

Book Reviews 320

Books Received for Review 325

PUBLISHED MONTHLY

THE JOHNS HOPKINS PRESS, Baltimore-18, Maryland

Made in United States of America

Copyright, 1949, by The Johns Hopkins Press

BULLETIN
OF
THE JOHNS HOPKINS HOSPITAL

MANAGING EDITOR, E. COWLES ANDRUS

ASSOCIATE MANAGING EDITOR, FREDERIK B. BANG

EDITORIAL BOARD

Charles R. Austrian — Edward M. Hanrahan
Kenneth C. Blanchard — John Eager Howard
Arnold R. Rich

All correspondence relating to the publication of papers should be addressed to The Editor, Bulletin of The Johns Hopkins Hospital, Baltimore-5, Md.

Books for review should be sent to The Editor at the above address.

Twenty-five reprints, without covers, of articles will be furnished to contributors free of cost. An order slip for additional reprints, with a table showing cost, is sent with proof.

Correspondence concerning business matters should be sent to The Johns Hopkins Press, Baltimore-18, Maryland, U. S. A.

This periodical is issued monthly. Two volumes a year are issued, each consisting of approximately 400 pages, and beginning in January and July, respectively.

Subscription price. In the United States and its possessions $6.00 per year. In Canada $6.25 per year; in foreign countries $6.50 per year. Prices for back volumes and back single numbers on application. Single numbers of the current volume, when available, $.75. Prices are net, postpaid.

New subscriptions and renewals are entered to begin with the first issue of the current volume. Should any issue of the current volume be out of print at the time the subscription order is received, the pro rata value of such numbers will be credited to the next volume, and the renewal invoice therefore adjusted accordingly.

Subscriptions should be renewed promptly. To avoid a break in your series, subscriptions should be renewed promptly. The publisher cannot guarantee to supply back issues on belated renewals.

Subscriptions, new or renewal, may be sent to the Publishers or ordered through your agent.

Subscriptions in the British Empire except Canada may be sent to: Baillière, Tindall & Cox, 8 Henrietta St., Covent Garden, London, W.C. 2.

THE JOHNS HOPKINS PRESS
BALTIMORE-18, MARYLAND, U. S. A.

Entered as second-class matter at the Baltimore, Md., Post Office. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

STUDIES OF RESPIRATORY AIR FLOW[1]

1. Significance of the Normal Pneumotachogram

DONALD F. PROCTOR and JANET B. HARDY

From the Departments of Otolaryngology and Pediatrics of the Johns Hopkins Medical School and the Johns Hopkins Hospital

Received for publication June 30, 1949

The series of investigations to be reported here and in succeeding papers is chiefly concerned with the exchange of gases between the alveolar atmosphere and the ambient air and with the pathological conditions affecting it. The frequency with which this phase of respiratory function is primarily affected by common pathological conditions and the general lack of information on the subject have been the stimulus for undertaking this work.

RESPIRATORY MECHANICS

The physical characteristics of the flow of air through the respiratory tract may be looked upon as reflections of the physiological apparatus producing and controlling this flow.

Air moves in or out of the lungs because of the existence of a pressure gradient between the lungs and atmosphere. The velocity of this flow is determined by the relationship between this pressure gradient and the resistance of the tract through which the air passes. This pressure gradient is ordinarily produced by the movements of the diaphragm and the thoracic cage (the force of these movements transmitted to the lungs through the pleural fluid). The elasticity of the lungs and thoracic wall and the intra-abdominal pressure add to or detract from the force of these movements. The movement of blood within the thorax or abdomen and the viscosity and inertia of the structures contained therein resist the forces exerted by the diaphragm, the intercostal muscles, and the elastic fibers of all of the involved structures.

[1] Work done with the cooperation of Dr. Ross McLean and the Respiratory Laboratory of the Department of Medicine of the Johns Hopkins Medical School, and the technical assistance of Mary Linderman.

This work has been supported by Grants in Aid from the U. S. Public Health Service.

The resistance to air flow offered by the respiratory tract is a function of the length, shape, and cross sectional area of the conducting tubes (1) (in part determined by the surrounding musculature, the thickness of the lining mucous membrane and the fluid lining the

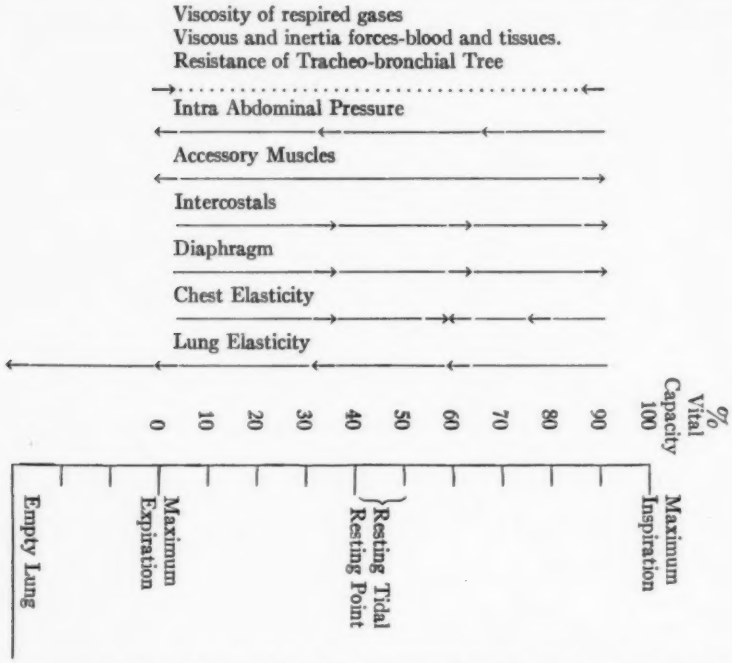


FIGURE 1. Forces involved in the dynamics of respiration. Note change in direction of force of chest elasticity at about 70% of vital capacity, Rahn et al.[19]

tubes). One might think that this resistance would be greater during expiration when the bronchial lumen is smaller, but the decreasing length of the bronchi which occurs simultaneously maintains a constant resistance during the entire respiratory cycle. These changes in the bronchi are easily observed during bronchoscopy and have been studied by other methods (2). The viscosity of the respired gases also plays a part in the resistance to their flow. (Fig. I).

The forces brought into play by the muscles involved, and the

rhythm at which they function are determined by a multitude of physiological controls including the autonomic nerve endings within the respiratory tract and elsewhere, the pressure in the carotid artery at the level of the carotid sinus, the O_2 and CO_2 tensions in the arterial blood, and other more or less well understood controlling factors.

TABLE I

Age distribution of subjects studied

	NORMALS	TOTAL
Birth to 24 hours	3	4
25 hours to 30 days	9	11
31 days to 60 days		2
61 days to 6 months	5	6
7 months to 1 year	1	3
13 months to 2 years	2	2
25 months to 9 years	2	12
10 years to 14 years		7
15 years to 20 years	3	6
21 years to 30 years	19	24
31 years to 40 years	13	22
41 years to 50 years	3	10
51 years to 60 years	1	6
61 years to 70 years		4
Total	61	119

THE PNEUMOTACHOGRAM

The pneumotachogram is the record obtained when one continuously measures (usually by a pressure gradient) the velocity of air flow, at the mouth or nose, during respiration, as a function of time.

Since 1925 when Fleish (3) first constructed a device for the continuous recording of the velocity of flow of respired air as a function of time, numerous investigators have attempted to analyse the pneumotachogram. Bretschger (4), Hartwich (5), Hamada (6), Rumpf (7) Gukelberger (8), Silverman (9, 10, 11), Kay (12), Cain (13), and many others have attempted to devise a means of using it to interpret the significance of pathological conditions involving the respiratory tract.

This report and those to follow comprise a study of 119 individuals, 61 normal persons and 58 patients with pulmonary disease. The age distribution is shown in Table I.

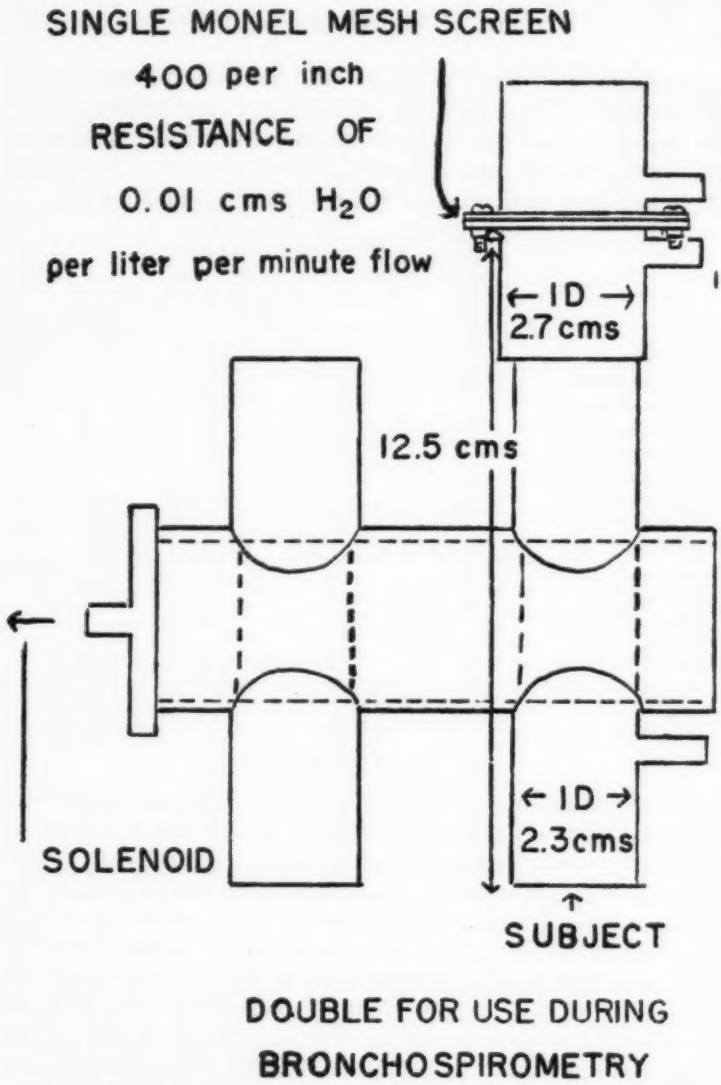


FIGURE II. Diagram of apparatus. Pneumotachometers have been calibrated with Schutte Koerting Rotameters. Dead space of apparatus is 69 cc. Responses to sudden pressure changes equal to those obtained in the studies reported occur in 0.04 seconds.

Figure II illustrates the apparatus now in use, the one used in most of the studies herein reported. In certain special experiments and in the earlier work somewhat different pneumotachometers were used.

TABLE II

Nomenclature for analysis of pneumotachogram

V_I	Zero velocity just before inspiration
V_E	Zero velocity just before expiration
V_{DP}	First point in inspiration when velocity increase in succeeding 0.1 seconds is less than $\frac{1}{3}$ velocity increase in the preceding 0.1 seconds.
V_{CP}	Point at which velocity decrease in succeeding 0.1 second is more than twice that in the preceding 0.1 second.
V_{EP}	Point of highest velocity during expiration.
T_R	Time from point V_I to V_I for next cycle.
T_I	Time from point V_I to V_E.
T_E	Time from point V_E to V_I for next cycle.
T_{IDP}	Time required to reach V_{DP} from V_I.
T_{ICP}	Time required to reach V_{CP} from V_I.
T_{EEP}	Time required to reach V_{EP} from V_E.
V_{RIP}	Peak inspiratory velocity.
V_{REP}	Peak expiratory velocity (same as V_{EP}).
V_{MIP}	Peak inspiratory velocity during maximum effort.
V_{MEP}	Peak expiratory velocity during maximum effort.
$\frac{V_{RIP}}{V_{RIA}}$	Peak inspiratory velocity ÷ average velocity for inspiration.
$\frac{V_{REP}}{V_{REA}}$	Peak expiratory velocity ÷ average velocity for expiration.
A_{IDP}	Slope of line from V_I to V_{DP} expressed as ccs per (0.1 sec.)^2
A_{CPE}	Slope of line from V_{CP} to V_E expressed as ccs per (0.1 sec.)^2
A_{EEP}	Slope of line from V_E to V_{EP} expressed as ccs per (0.1 sec.)^2
A_{EPI}	Slope of line from V_{EP} to V_I expressed as ccs per (0.1 sec.)^2
$\frac{T_R}{T_I}$	Total cycle time ÷ inspiratory time.
$\frac{V_{MIP}}{V_{RIP}}$	Inspriatory maximum effort peak velocity ÷ resting inspiratory peak velocity.
$\frac{V_{MEP}}{V_{REP}}$	Expiratory maximum effort peak velocity ÷ resting expiratory peak velocity

The pressure drops have varied from the highest of 0.03 to the lowest of 0.007 cms. H_2O per 1 liter per minute flow.

In order to discuss the pneumotachogram it is necessary to use an intelligible nomenclature. The one used in this paper with appropriate definitions is to be found in Table II.

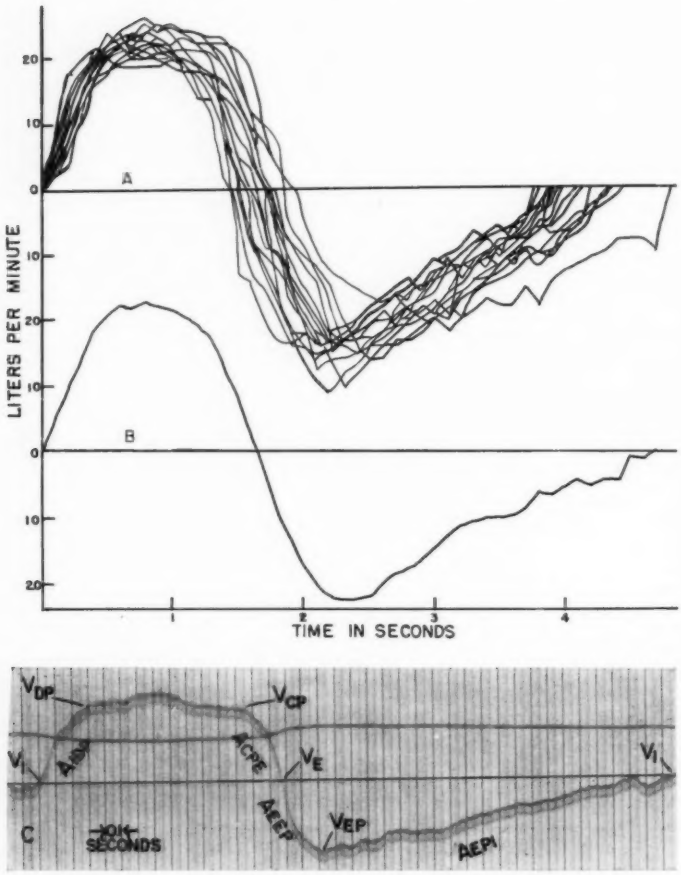


FIGURE III-A. Sixteen consecutive cycles, superimposed, on one normal subject.
B. Mean of the above sixteen cycles.
C. Record of one cycle. Time lines 0.01 seconds. (In all other records time lines represent 0.1 second). Inspiration above zero line.

Comparison of consecutive cycles or cycles taken on different days from records on any single subject impresses one with the consistency with which an individual pattern is reproduced. (Fig. III)

That different individuals have widely differing patterns is easily

seen when one tries to superimpose any subject's pattern on that of another subject. (Fig. IV)

If one attempts to imitate the pattern of another subject he readily sees that these patterns are either the result of deeply seated habit or of relatively stable characteristics of the individual respiratory system.

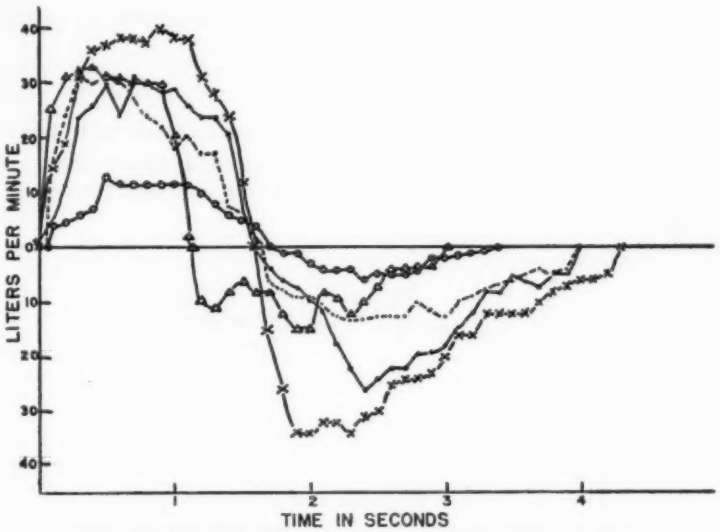


FIGURE IV. Superimposition of cycles from five normal subjects.

ANALYSIS OF THE PNEUMOTACHOGRAM

Certain conclusions, founded on a priori reasoning and borne out by experimental observations, may be drawn from consideration of the subject whose mean pneumotachogram is shown in figure III.

1. Action of the inspiratory musculature

The initial rise to peak velocity A_{IDP} represents the movement of the diaphragm supplemented by beginning movement of the chest wall. Records taken while mechanically limiting the motion of the abdomen show a partial elimination of the point V_{DP} and a change in the initial rising line to approach one such as A_{ICP}. (Fig. V)

The point V_{CP} represents the peak action of the intercostals supplemented by continuing but slowing motion of the diaphragm. When the movement of the chest wall is mechanically limited the pattern approaches that one might see in A_{IDP} and A_{DPE}. (Figure V)

2. Transition from inspiration to expiration

Inspiratory effort is terminated rather suddenly as seen by the slope A_{CPE}. In studying the pneumotachogram it is important to realize

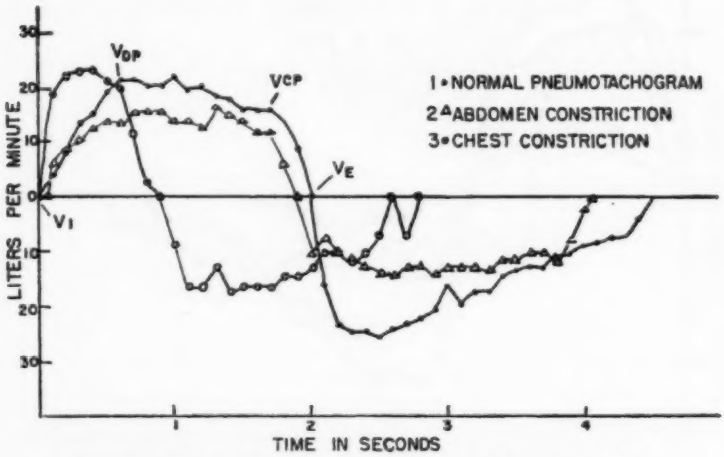


FIGURE V. Effects of mechanical limitation of abdomen and chest.

that at the point V_E air has just ceased to move into the lungs and is just beginning to move out. The action of the inspiratory muscles probably begins to be inhibited shortly after the point V_{CP} but it is likely that all inspiratory effort is not stopped until shortly after V_E. The reversal in direction of flow occurs when the elastic forces exceed the subsiding force of inspiratory effort.

Under ordinary conditions the sharp slope of the line crossing zero at V_E is indicative of the fact that the termination of inspiratory effort is a relatively rapid procedure. This phenomenon may be compared to the archer drawing a bow string. He draws the bow string back, at first rapidly, then more slowly. When it is all the way back,

with excellent muscular coordination, the archer pauses for a moment to take aim. It is exactly this pause which is ordinarily lacking in respiration. The bow is drawn and released automatically at a given point of tension by a neat trigger mechanism, sometimes spoken of as the Hering-Breuer reflex.

3. Action of lung elasticity in expiration

Since the continuing force of inspiratory effort beyond the point V_E is probably negligible and since expiration involves no muscular activity the slope A_{EEP} results from the simple opposition of the following forces:

Resistance to airflow and resistance to thoracic and abdominal tissue distortion ——— Elastic tension at given degree of inflation.

The peak velocity V_{EP} is a measure of lung elasticity when considered in conjunction with:

a. The pressure gradient associated with such a flow velocity in the individual.

b. The volume of inflation of the lungs beyond the resting point of the chest.

c. The resistance offered by other viscous and inertia forces.

The slope A_{EPI} is a measure of the decrease in flow velocity concomitant with the change in lung elasticity with decreasing volume. The fact that fall in velocity along this line is almost linear in respect to time indicates that expiration is terminated at the resting volume of the chest (the point at which lung elasticity and chest elasticity are balanced).

A demonstration of the effect of elasticity on the pneumotachogram is accomplished by two simple experiments. When a subject increases his inspiratory volume there is a corresponding increase in the height of the point V_{EP} and a sharper slope to A_{EEP} in the ensuing expiration. (Fig. VI-A) When the subject stops at the end of a normal expiration and breathes out an additional tidal volume, then relaxes for inspiration (allowing chest elasticity to restore the thorax to its resting position) one readily sees the inspiratory pattern assuming the shape of the normal expiratory pattern. (Fig. VI-B)

Twenty of the normal adult subjects (52%) had patterns similar to that shown in Figure III. In nine (24%) the patterns were quite

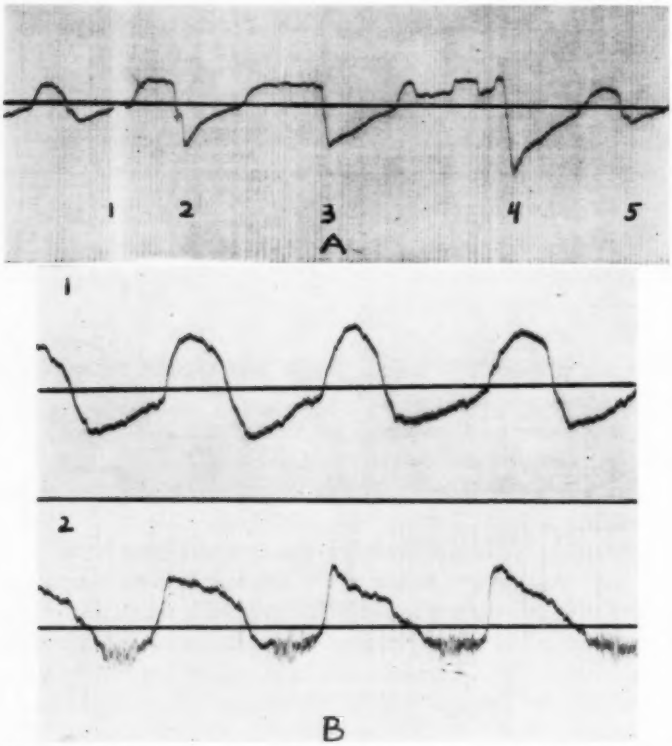


FIGURE VI-A. Increasing inspiratory volumes beginning at 1; 2, 3, & 4 expiratory peaks following larger inspirations; 5 return to normal.

FIGURE VI-B. Breathing below resting point of chest 2, compared to normal pneumotachogram on same subject 1.

similar except for the termination of expiration. In these subjects the slope A_{EPI} is terminated by an almost vertical line going from flows of 5-20 liters per minute to zero in 0.1 seconds. (Fig. VII-A) This probably signifies either a failure to obtain a true resting record, or an habitual breathing at a volume slightly above the normal resting

point of the chest. The remaining nine subjects (24%) show a very different pattern in which there are no sudden changes in velocity (note the slow cross-over at V_E) peaks are relatively low, and expira-

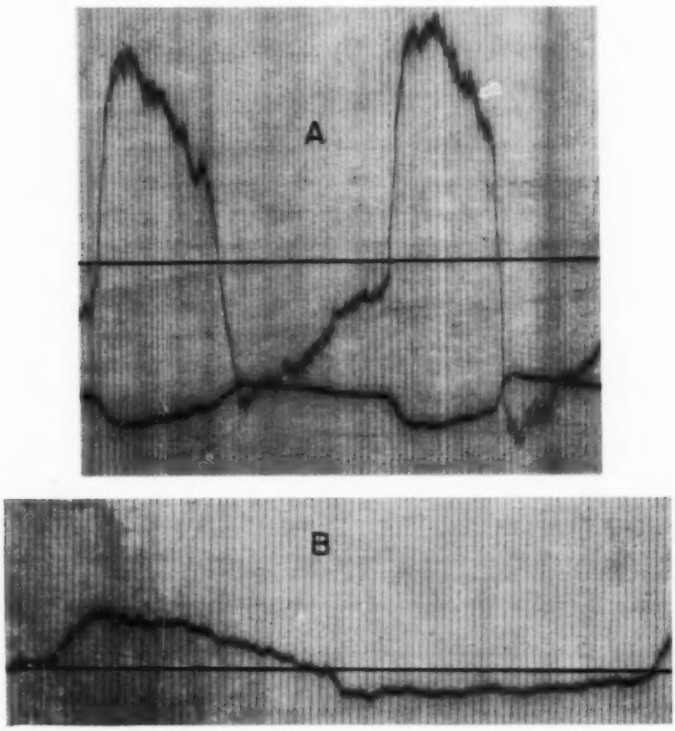


FIGURE VII-A. Second type of normal pneumotachogram.
B. Third type of normal pneumotachogram.

tion and inspiration more closely resemble one another. (Fig. VII-B) The significance of this type of pattern has not been determined.

No normals in this series have consistently had post-inspiratory pauses (periods of zero flow of appreciable length), but several have had post-expiratory pauses. These appear as a perfectly flat line on zero for 0.1 to 0.4 seconds at the end of expiration, or a zero line punctuated by a brief expiratory or inspiratory puff of low velocity.

Such a pause occurring in one of our normal subjects showed a change in character at a later date (during the seventh month of pregnancy) (Fig. VIII)

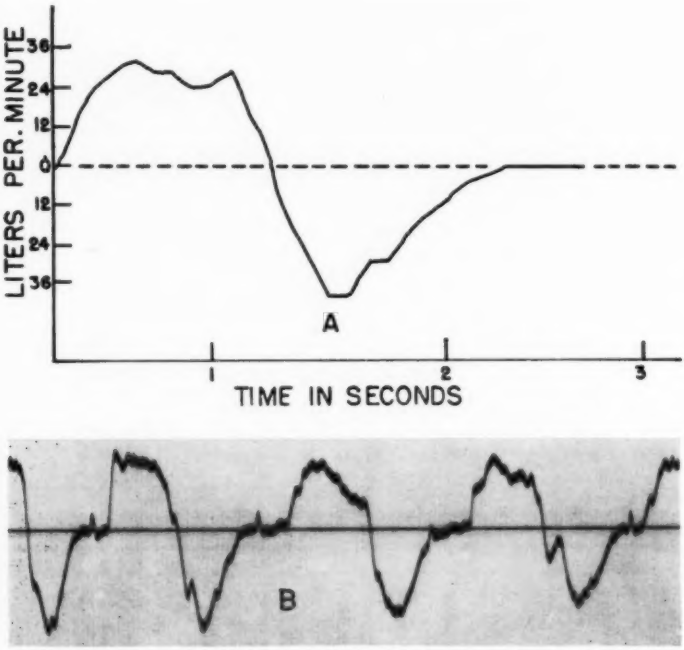


FIGURE VIII-A. Before pregnancy.
B. During seventh month of pregnancy.

Hyperventilation will produce a post expiratory pause in a normal subject not ordinarily having one. It is conceivable that when it occurs regularly the subject is one who, in one sense of the word, chronically hyperventilates.

In figure IX-A is shown a record taken on a normal 24 hour old infant. The peak flows are between 2 and 3 liters per minute; tidal volume is a little over 10 ccs; respiratory rate is a little over 40. The infant patterns, (much more widely varied in shape than the adult), at times are quite repetitive. Figure IX-B shows a record taken at the beginning

of a crying spell in a 16 day old premature infant (1800 grams at birth). Other studies of infants and children will be reported in a later paper.

The effects of position sleep, exercise, etc. have not as yet been adequately explored. Pneumotachograms taken under such circumstances on one normal subject are illustrated in Figure X.

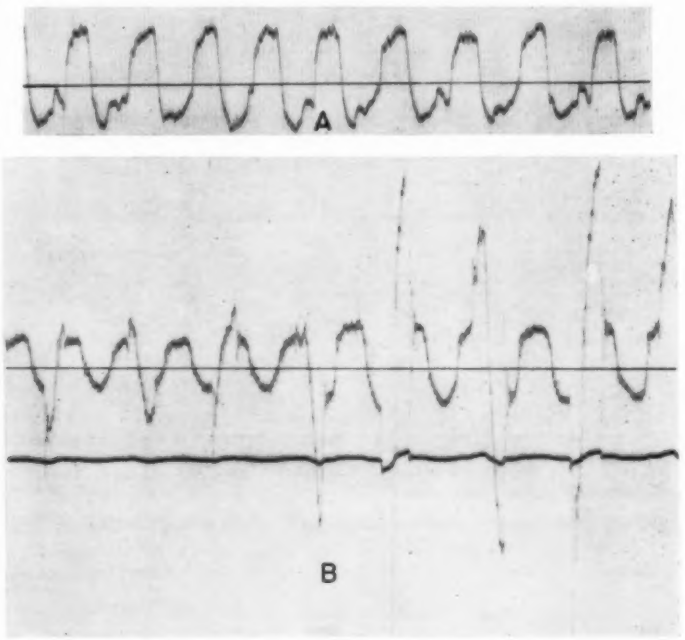


FIGURE IX-A. Normal 24 hour infant.
B. Crying spell in 16 day old premature infant.

QUANTITATIVE ANALYSIS

An attempt has been made to submit the pneumotachograms studied to various forms of quantitative analysis. Bretschger's (4) division of all patterns into three groups according to their approach to the shape of a dome, plateau, or triangle seems to be unsatisfactory. The majority of the patterns in this study fit only very roughly into such categories. Gukelberger's (8) suggestion that the rates of acceleration

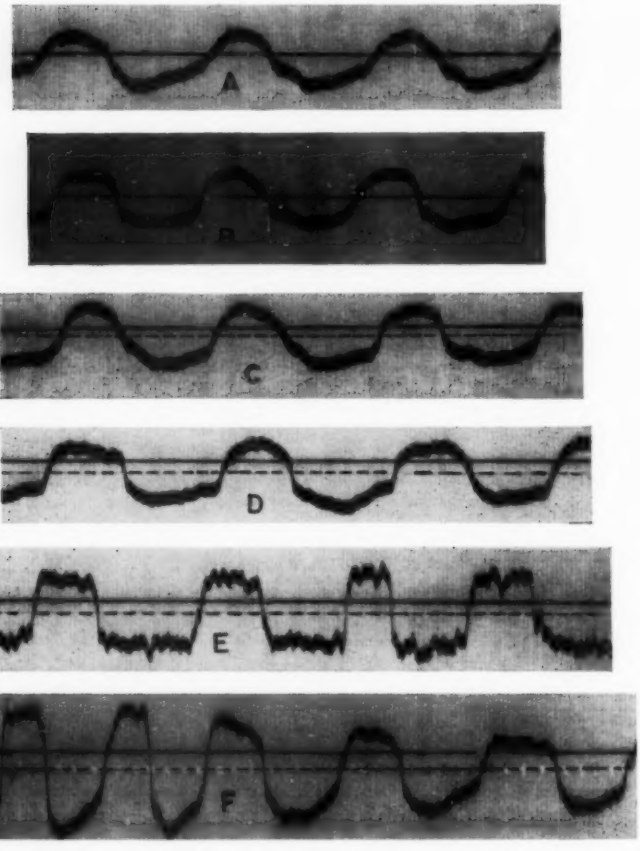


FIGURE X. Pneumotachograms during: a. sitting, b. standing, c. lying supine, d. lying prone, e. during moderate exercise of legs, f. after severe exercise.

Zero passes along top of record in a; in all others it passes through center of record line. In c, d, e & f zero line is dotted.

and deceleration be studied is useful but does not consider other significant characteristics. The same objections hold for the methods of analysis used by Silverman and others.

Studies of the subjects herein reported include measurements of

acceleration, velocity and time relationships at various points during the cycle.

Patterns which seem quite similar in shape, when submitted to such methods of quantitative analysis yield widely differing figures. Patterns in patients with pulmonary disease (to be reported in a forthcoming paper), with obvious variations from the normal configuration, break down into figures which often lie in the range of normal and, even in extreme instances, are not significantly different from those obtained from the normals. As examples some of the figures obtained are listed in Table III.

TABLE III

Quantitative analysis of pneumotachograms

(For definitions see Table II)

	AVERAGE NORMAL	NORMAL RANGE	RANGE IN PULMONARY DISEASE (24 PTS.)	NUMBER WITH PULMONARY DISEASE OUTSIDE OF NORMAL RANGE
T_R/T_I	2.22	1.57– 2.90	2.07– 3.33	2
A_{EEP}*	12.00	1.43–45.30	2.30–48.00	1
A_{IDP}*	20.00	4.00–72.00	2.50–97.00	5
V_{RIP}†	35.00	13.00–78.00	—	—
V_{REP}†	29.00	6.00–67.00	—	—
$\frac{V_{RIP}}{V_{REP}}$	1.29	0.73– 2.33	0.73–2.27	0
$\frac{V_{RIP}}{V_{RIA}}$	1.55	1.27– 1.86	1.18– 2.85	8
$\frac{V_{REP}}{V_{REA}}$	1.70	1.15– 2.88	1.17– 2.50	0
$\frac{V_{MIP}}{V_{RIP}}$	5.00	2.50– 7.55	1.25– 6.15	7
$\frac{V_{MEP}}{V_{REP}}$	6.33	1.40–13.30	1.18– 4.25	2

* ccs. per (0.1 second)^2.

† Liters per minute.

Possible explanations for the failure of such an analysis to yield significant similarities or differences may lie in the following hypotheses:

1. The records studied may not represent true resting pneumotachograms.

2. The methods of quantitative analysis may not include the fundamental characteristics of the pattern. Perhaps an analysis of the total shape of the curve is required.

3. Some supposedly normal subjects may not have completely normal respiratory systems or respiratory habits.

4. The pneumotachogram may not be a consistently reliable method for study.

5. As in other studies of respiratory function the most useful interpretations may be made by correlating the findings in a variety of functional tests. A comparison of function when the subject is at rest with the subject during maximum effort, may be especially useful.

MAXIMUM EFFORT PNEUMOTACHOGRAM

In an attempt to explore the last of these hypotheses several methods of analysing maximum effort have been tried. Heretofore such studies have been largely confined to the performance of the standard "maximum breathing capacity" test: the attempt to produce, against low resistance, maximum ventilation of the lungs over a period of twenty seconds expressed in terms of liters per minute.

When one attempts to concentrate maximum effort on one expiration or inspiration, peak flows of over 250 liters per minute can be reached in 0.1 second on expiration and a little below 200 liters per minute in 0.23 seconds on inspiration. The slope of the expiratory line is almost perfectly straight whereas the inspiratory slope curves sharply at the top. (Fig. XI)

If no other limiting factors were involved, such a subject should be able to perform a maximum breathing capacity with a cycle of 0.33 seconds regardless of the tidal volume resulting. Actually in this subject when the cycle length was 0.33 seconds during such a test the tidal fell below 200 ccs. and the ventilation was well below half the maximum breathing capacity for the individual.

When tidal volumes, during maximum breathing capacity tests are charted against the length of the cycle a straight line relationship is found; but this line does not pass through zero. Instead it crosses the time line at about 0.13 seconds. (Figure XII-A) Approximately

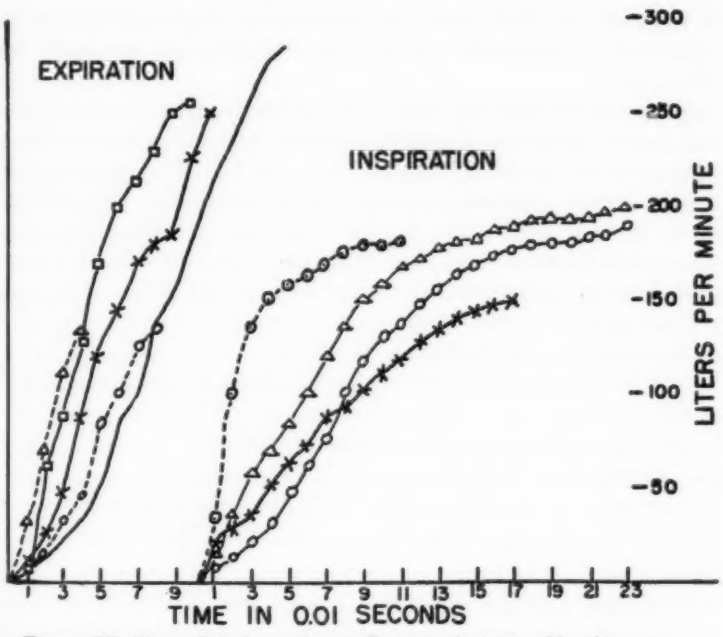


FIGURE XI. Chart of single maximum efforts, expiratory and inspiratory.

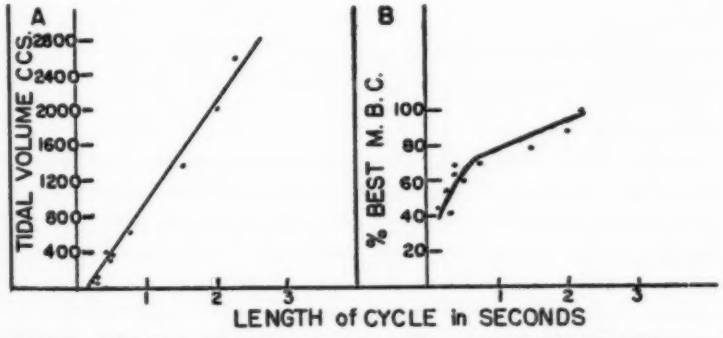


FIGURE XII-A. A chart of tidal volume against cycle length during performance of maximum breathing capacity tests.

B. Maximum breathing capacity charted against cycle length.

this amount of time seems to be required for the neuromuscular apparatus to stop motion in one direction and start motion in the other. (14)

If maximum breathing capacity is charted against length of cycle one finds a sharp rise in ventilation with increasing length of cycle until about 0.6 seconds is reached. (Figure XII-B) At about this point the line breaks and increase of ventilation is much less with each increase in cycle length. The highest maximum breathing capacity in this normal subject was obtained with a cycle of 2.2 seconds. Ordinarily peak flows can only be maintained for about 0.7 seconds in inspiration and 1.0 seconds in expiration.

These figures may be restated as follows:
Length of cycle for maximum breathing capacity in this normal should equal:
0.1 seconds: time to reach peak expiratory flow
0.23 seconds: time to reach peak inspiratory flow
0.13 seconds: time to change direction at the end of inspiration
0.13 seconds: time to change direction at the end of expiration

0.59 seconds.

But one must also consider the facts that, on the one hand, the mere change in direction of flow means waste time; and, on the other hand, peak flows can only be maintained for certain intervals. Therefore we add to
0.59 seconds
1.0 seconds: maintenance expiratory peak
0.7 seconds: maintenance inspiratory peak

2.29 seconds

It will be readily seen that these theoretical considerations have led to the first value of 0.59 seconds which coincides with the break in the curve in figure XII-B and 2.29 seconds which is very near the cycle length actually employed by this subject during his most successful effort.

Many poorly controlled factors enter into the ordinary test for maximum breathing capacity: the resistance of the apparatus, the subject's willingness to cooperate: his cleverness of performance, the tendency to choke and cough, etc. It seems reasonable to suggest that a

better understanding of the information sought could be obtained by the study of the maximum effort pneumotachogram and alveolar pressures.

The ability of the subject to reach peak velocities, the pressure gradients associated with such velocities, and other physical factors controlling the subject's ability to produce high rates of ventilation should all be studied for a more complete understanding of his ventilatory reserve.

In the normal subject maximum effort pneumotachograms consistently reveal rapid rates of acceleration and deceleration at the beginning and end of inspiration and expiration, a triangular pattern when the emphasis is placed on rapidity, and a nearly rectangular one when the emphasis is on depth. (Figure XIII)

Peaks reached during maximum effort tend to be much higher than those reached in quiet breathing. On the average among the normal subjects studied maximum inspiratory peaks were slightly over five times as great as resting inspiratory peaks whereas maximum expiratory peaks were well over six times higher than resting expiratory peaks.

On the average the ratio between resting inspiratory peaks and expiratory peaks was $V_{RIP}/V_{REP} = 1.29$.

ADDED RESISTANCE TO BREATHING

Measurement of alveolar-atmosphere pressure gradients is of great importance in evaluating the role of resistance to the flow of air in respiration. Such measurements have been made on all of the subjects studied in the manner reported by Otis and Proctor. (15) The method is based on that first used by Vuilleumier (16) the principle of which (along with a sample record) is shown in figure XIV.

The simplest example of added resistance to breathing is the change from mouth to nasal breathing. In the normal subject such a change can add sufficient resistance to produce alveolar pressure gradients as much as five times greater than those in mouth breathing.

Studies of the effect of added resistance to breathing have been so far confined to one normal subject. The lowest resistance used, 0.1 cms. H_2O per liter per minute flow, is comparable to the resistance of nasal breathing. Other added resistances produced pressure drops

of 0.15, 0.36, and 0.5 cms. H_2O per liter per minute flow. These were all compared to the resistance of the pneumotachometer of 0.01 cms.

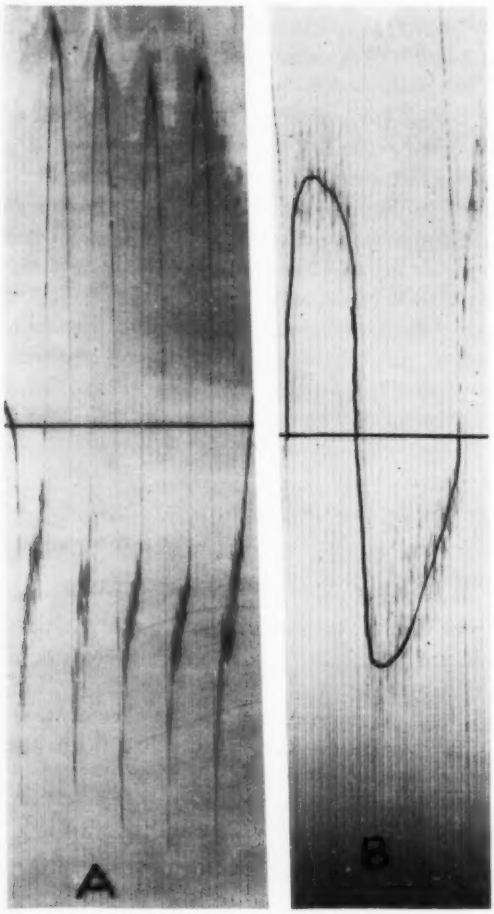


FIGURE XIII. Maximum effort pneumotachogram (a) rapid and (b) deep

H_2O per liter per minute flow. (Figure XV-A) Records were taken when the subject felt that respiration was quiet (usually about 30 seconds

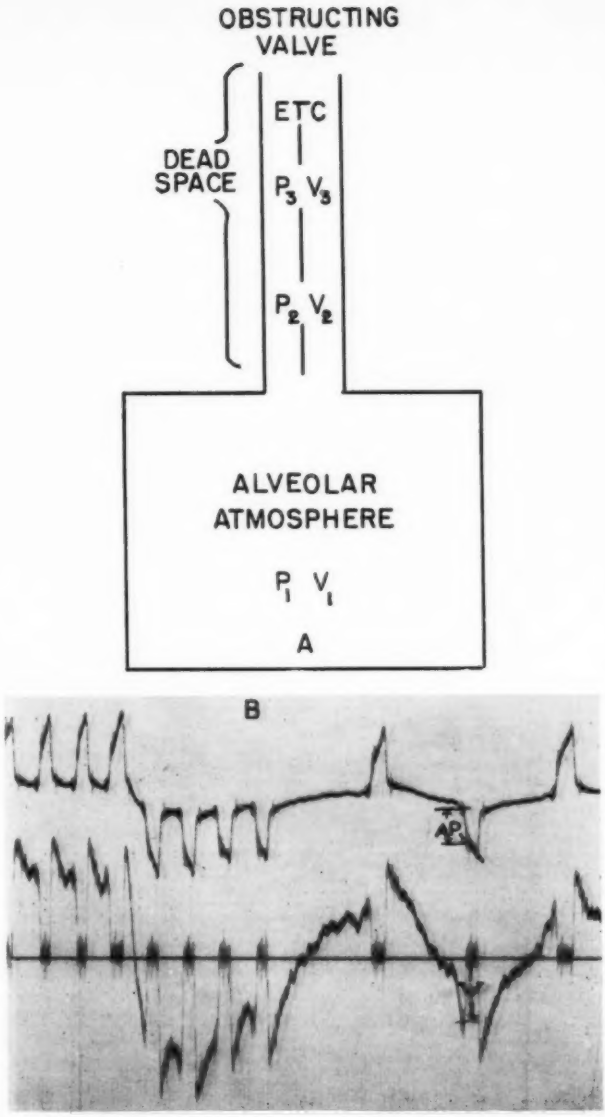


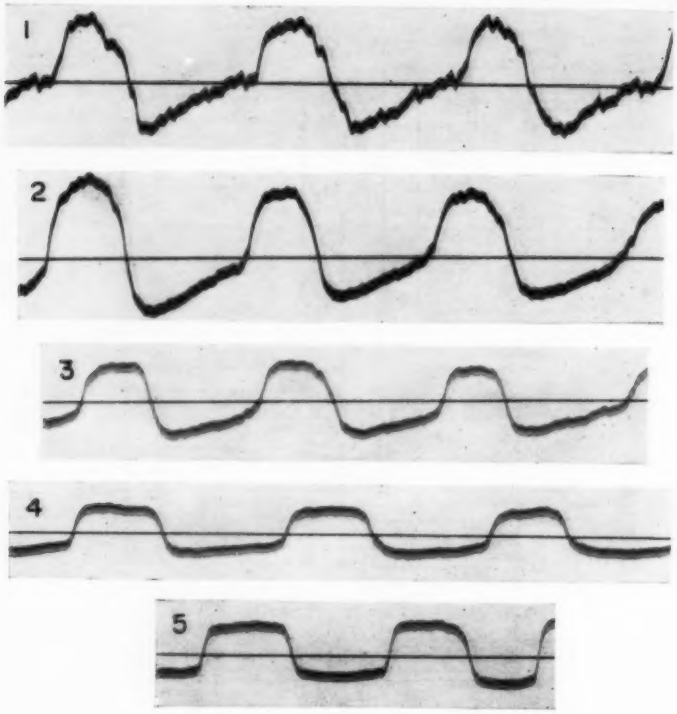
FIGURE XIV. Principle of measurement of the alveolar pressure gradient by method of Vuilleumier and record with pneumotachogram.

A. Sudden obstruction to air flow at the mouth results in rapid equalization of pressure throughout the respiratory tract.

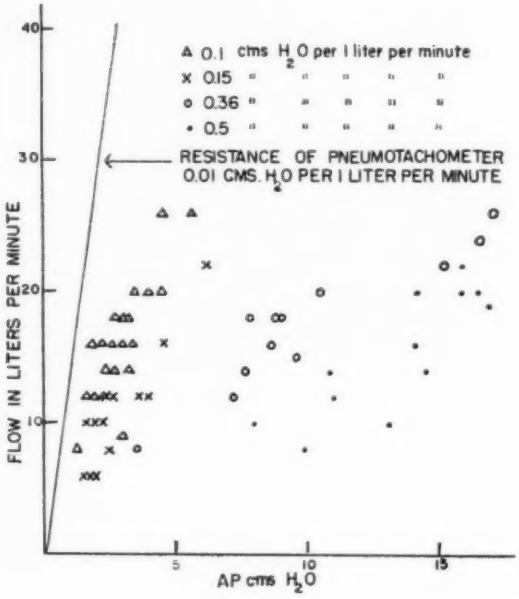
The large alveolar volume in comparison to dead space volume causes this equalized pressure to closely approximate alveolar pressure at moment of interruption.

PV (equalized pressure and total respiratory volume) = $P_1V_1 + P_2V_2 + P_3V_3 +$ etc.

B. Alveolar pressure record (above) and pneumotachogram (below).


1
2
3
4
5

A


Δ 0.1 cms H2O per l liter per minute
x 0.15 " " " " "
o 0.36 " " " " "
• 0.5 " " " " "
RESISTANCE OF PNEUMOTACHOMETER
0.01 CMS. H2O PER I LITER PER MINUTE
FLOW IN LITERS PER MINUTE
40
30
20
10
5
10
15
ΔP cms H2O

B

FIGURE XV

after starting to breathe through the apparatus). The highest resistance was sufficient to cause definite discomfort to the subject who was seated and at rest.

It will be noticed that the lowest resistance added alters only slightly the normal respiratory pattern whereas the additional resistances produce patterns with an almost rectangular wave, expiration being very similar in appearance to inspiration. In conjunction with this the subject noted a necessity for employing the respiratory muscles in expiration as well as in inspiration and a conscious desire to end each phase of each cycle and begin the next.

With the highest resistance tidal volume is higher by 14%, respiratory rate more rapid by 40% and the proportion of the cycle spent on expiration is less in comparison to the no resistance pattern by 10%. (Tidal volumes without the resistance were about 500 ccs.) The lesser degrees of resistance showed tidals smaller, respiratory rates only slightly higher and proportion of time spent on expiration slightly higher than normal.

The alveolar pressure gradients accompanying these resistances are shown in Figure XV-B. It would seem that, when resistances are such as to increase the alveolar pressure gradient by a factor of five or less, relatively little change in respiratory activity occurs. An increase by a factor of ten or more produces changes involving increase in minute ventilation and almost completely active expiration.

Of greater practical importance is the radical curtailment in the ability of the individual to produce increases in ventilation with maximum effort. (Figure XVI)

Many aspects of this subject have been explored by Silverman (17, 18) and more recently by Cain (13).

In 1946 Rahn et al. (19) studied the pressure volume diagram of the chest. This work has been repeated using the pneumotachogram for

FIGURE XV-A. Pneumotachograms on normal subject

1. Added resistance of pneumotachometer alone (0.01 cms. H_2O per 1 Lit/Min flow)
2. Added resistance of 0.1 cms. H_2O per 1 Lit/Min flow.
3. Added resistance of 0.15 cms. H_2O per 1 Lit/Min flow.
4. Added resistance of 0.36 cms. H_2O per 1 Lit/Min flow.
5. Added resistance of 0.5 cms. H_2O per 1 Lit/Min flow.

B. Corresponding alveolar pressure gradients.

volume measurements and peak flows after release of obstruction, and the alveolar pressure interrupter for relaxation pressures. The subject would breath in or out any given quantity of air and then be obstructed. He would relax against the obstruction which would then be suddenly

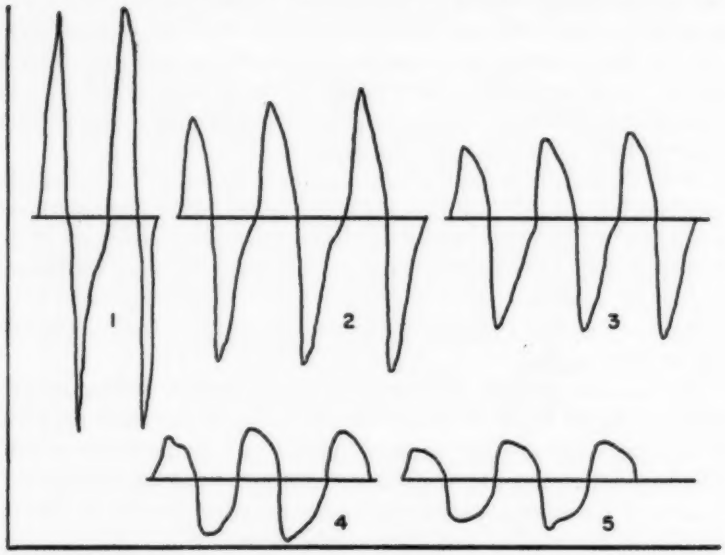


FIGURE XVI. Maximum effort pneumotachograms under circumstances of resistance listed in Figure XV-A, 1,2,3,4,5.

removed. In Figure XVII are shown the figures obtained and charted across them is the curve obtained by Rahn et al.

The pneumotachogram can also be employed in conjunction with a variety of other studies as an aid in the orientation of separate measurements in respect to time. (Figure XVIII-A & B) Charting the velocity of flow of respired air against such measurements as intrapleural presure, volume displacement, acceleration, lung elasticity etc. aids in the proper interpretation of these values (20, 21).

SUMMARY

1. Experimental analysis of normal pneumotachograms has substantiated the hypothesis that portions of the respiratory pattern are

true reflections of the activity of the respiratory musculature, the effectiveness of lung elasticity, and the manner in which reversal of respiratory effort is accomplished.

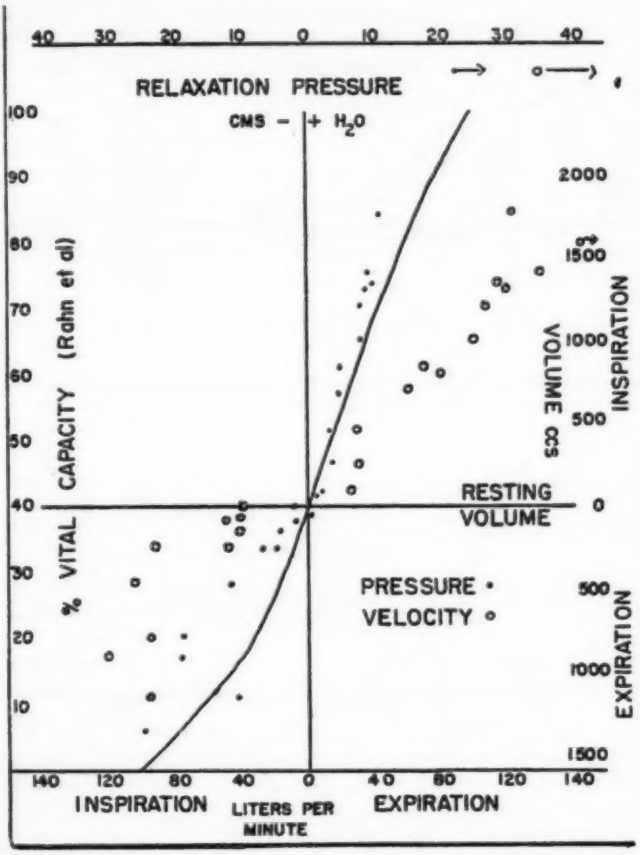


FIGURE XVII. Pressure volume diagram of the chest. The solid line is taken from Rahn et al (17).

2. Study of records taken during maximum respiratory effort provides information facilitating a classification of the factors controlling the ventilation reserve: the ability rapidly to reach high flow velocities,

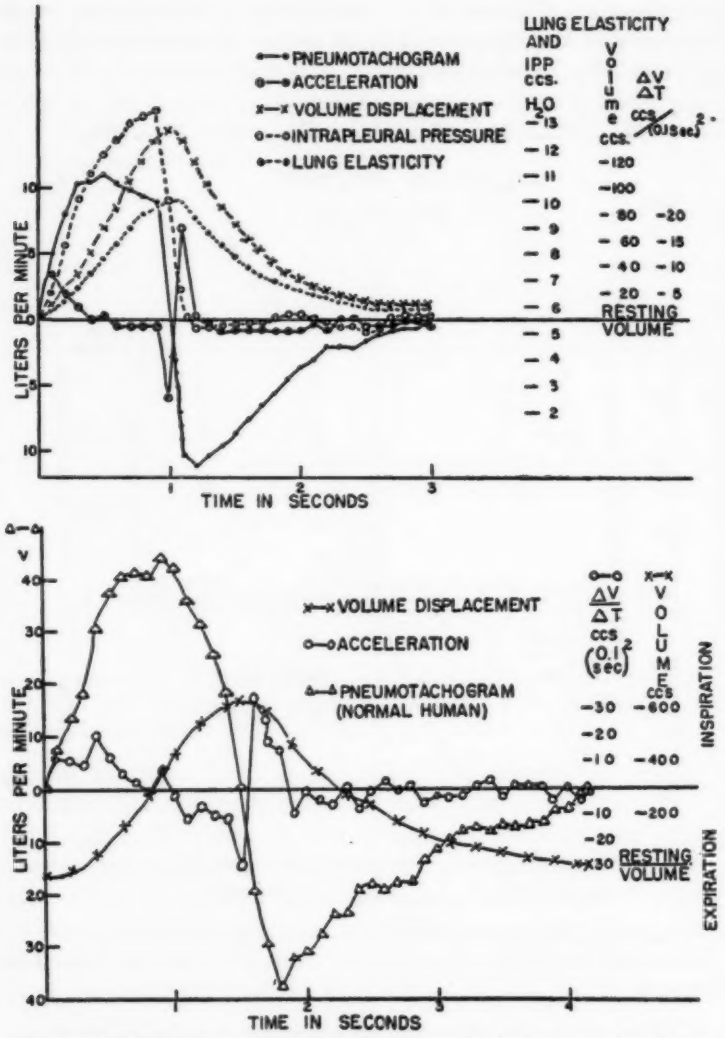


FIGURE XVIII-A. Intrapleural pressure, pneumotachogram, acceleration, volume displacement, elastic tension (calculated). Measurements taken on dog under nembutal anaesthesia.

B. Pneumotachogram, acceleration, and volume displacement on human subject.

the time during which such high flow velocities can be maintained, and the time required for reversal of direction of flow of air.

3. The pneumotachogram yields information about the manner in which the organism adapts the respiratory apparatus to added resistance to breathing, especially when combined with studies of the alveolar-atmosphere pressure gradient.

BIBLIOGRAPHY

1. Rohrer, Fritz—Physiologie der Atembewegung. Handbuch der Normalen und Pathologischen Physiologie, pps. 70–127 Zweiter Band, 1925.
2. Ellis, M.—The Mechanism of the Rhythmic Changes in the Calibre of the Bronchi During Respiration. J. Physiol. **87:** 298–309, 1936.
3. Fleisch, A.—Der Pneumotachograph; ein Apparat zur Geschwindigkeitsregistrierung der Atemluft. Pflüger's Arch. f.d. ges. Physiol. **209:** 713–722, 1925.
4. Bretschger, H. J.—Die Geschwindigkeitskurve der menschlichen Atemluft. Pflüger's Arch. f.d. ges. Physiol. **210:** 134–148, 1925.
5. Hartwich, A.—Pneumotachographische Untersuchungen über die Atemverhaltnisse bei Hyper-und Dyspnoischen. Zeits. f.d. ges. Fxp. Med. **69:** 482–513, 1930.
6. Rumpf, K.—Pneumotachographische Untersuchungen an Gesunden, Emphysematikern und Herz Kranken im Bad. Zeits. f.d. ges. Exp. Med. **101:** 492–501, 1937.
7. Hamada, T.—Pneumotachographische Studien. Acta Scholar Medicinalis Universitalis Imper. in Kioto **16:** 47–98, 1933.
8. Gukelberger, N.—Zur Analyse des Pneumotachograms zur Dynamik der Atembewegung unter Normalen Bedingungen. Zeits. f.d. ges Exp. Med. **113:** 737–754, 1944.
9. Silverman, L., Lee, R. C. and Drinker, C. K.—A New Method for Studying Breathing, with Observations upon Normal and Abnormal Subjects. J. Clin. Invest. **23:** 907– 1944.
10. Silverman, L.—Respiratory Air Flow Characteristics and their Relation to Certain Lung Conditions occurring in Industry. Journ. Ind. Hyg. and To. **28:** 183–196, 1946.
11. Rao, M. N. and Silverman, L.—Respiratory Patterns in Pulmonary Tuberculosis. Am. Rev. Tbc. **54:** 574–581, 1946.
12. Kay, R., Whittenberger, J. L., Silverman, L. Respiratory Air Flow Pattern in Children. Am. J. Diseases of Children. V. 5 p. 625 May 1949.
13. Cain, C. C. and Otis, A. B.—Some Physiological Effects Resulting from Added Resistance to Respiration. Journal of Aviation Medicine (in press).
14. Otis, A. B. and Bembower, W. C.—Factors Related to Voluntary Ventilation Capacity. Proceedings Am. Physiol. Soc. V. 155, No. 3, Dec., 1948.
15. Otis, A. B. and Proctor, D. F.—Measurement of Alveolar Pressure in Human Subjects. Am. Jour. Physiol. **152:** 106–112, 1948.

16. VUILLEUMIER, P.—Über eine Methode zur Messung des intraalveolären Druckes und der Strömungswiderstande in den Atemwegen des Menschen. Zeits. Klin. Med. **143:** 698, 1944.
17. SILVERMAN, L., LEE, G., PLOTKIN, T., AMORY, L., AND YANCY, A.—Inspiratory and Expiratory Air Flow Measurements on Human Subjects with and without Resistance at Several Work Rates. O.S.R.D. Report, 1945, 33 pages.
18. SILVERMAN, L., LEE, R. C., LEE, G., DRINKER, K. R., AND CARPENTER, I. M.—Air Flow Measurements of Human Subjects with and without Resistance. O.S.R.D. Report, 1943, 122 pages.
19. RAHN, H., OTIS, A. B., CHADWICK, L. E. AND FENN, W. O.—The Pressure Volume Diagram of the Thorax and Lung. Am. Jour. Physiol. **146:** 161–178, 1946.
20. NEERGAARD, K. V. AND WIRZ, K.—Die Messung der Strömungswiderstande in den Atemwegen des Menschen, insbesondere bei Asthma und Emphysem. Zeitschr. Klin. Med. **105:** 52–82, 1927.
21. CHRISTIE, R. V.—Properties of the Emphysematous Lung and their Significance. Jour. Cl. Invest. **13:** 295–321, 1934.

HISTOCHEMICAL STUDIES ON CARTILAGE AND BONE
I. The Normal Pattern[1, 2]

RICHARD H. FOLLIS, JR., and MORGAN BERTHRONG

From the Department of Pathology, Johns Hopkins University School of Medicine

Received for publication, July 1, 1949

The histochemical observation that cartilage cells contain glycogen was made almost a hundred years ago. In the intervening years there have been numerous isolated studies applying chemical reactions to demonstrate other constituents in sections of cartilage and bone. No systematic or integrated investigations have been reported, however, nor have adequate supervital techniques been applied. Inasmuch as knowledge of the biochemical reactions as well as the chemical structure of normal cartilage and bone is so meager, we have thought that it would be desirable to apply certain qualitative techniques to these tissues in order to obtain an overall picture of the normal pattern which could then be used as a base line in an investigation of certain abnormal states produced by one means or another, with particular stress on vitamins and hormones. The present paper serves as a review and, in addition, will present certain unreported observations which have been carried out on the cartilage and bone of normal rats and guinea pigs and humans at autopsy.

No details of the methods used will be presented here. A description of or reference to each will be found in its appropriate place.

ENZYMES

Cytochrome Oxidase: In 1885 Ehrlich (11) initiated experimental intracellular chemistry when he noted the development of a blue color in many tissues of animals which had been injected with alpha naphthol and dimethylparaphenylendiamine, now called the nadi reagent. An enzyme, first named indophenol oxidase, was shown to be responsible

[1] A part of the observations herein presented were reported at the annual meeting of the Association of American Pathologists and Bacteriologists, March, 1948 (14).

[2] Aided by a grant from Mead Johnson and Company.

in the presence of oxygen, for the formation of the indophenol blue dye as a result of the oxidation of dimethylparaphenylendiamine and its reaction with alpha naphthol. More recently, evidence has accumulated that indophenol oxidase is cytochrome oxidase. The oxidation of the nadi reagent with the production of indophenol blue may therefore be used as a qualitative test for the presence of cytochrome oxidase in tissues.

In studying this reaction we have used thin, free hand slices of cartilage and bone, usually the costochondral junction or upper epiphysis of the tibia of rats and guinea pigs. Frozen sections, though of course thinner, do not give as active preparations. The tissue is placed in a drop of Krebs-Ringer phosphate solution (pH 7.4) on a slide and to this are added one or two drops of a freshly prepared solution of equal parts .01 M alpha naphthol and .01 M dimethylparaphenylendiamine (paraaminodimethylalanine) in Krebs-Ringer phosphate solution (pH 7.4). The tissue without a cover glass is then observed under low power of the microscope. Incubation hastens the reaction but is not obligatory. Certain adjunct procedures are necessary as controls. Since cyanide inhibits cytochrome oxidase activity, a drop of KCN (.01 or .001 M in Krebs-Ringer phosphate) is added just before the tissue is brought into contact with the nadi reagent. In addition, as Flexner (13) has suggested, ferricyanide should be added to the washed slice after the maximal development of blue color in order to determine whether the reagent has actually entered the tissue. Ferricyanide, of course, oxidizes the nadi reagent to indophenol blue.

When the nadi reagent comes in contact with slices of cartilage and bone, there is an almost immediate appearance of deep-blue staining granules about many of the bony trabeculae. Particularly prominent are aggregations close to the cartilage-shaft junction. These blue pigment granules are concentrated about the nuclei of osteoblasts and the few osteoclasts which may be present. The development of the color is, as noted, extremely rapid and is accelerated if a stream of oxygen is played over the slice. This intense coloration appears often before any very prominent blueing can be observed in striated muscle fibers which may be present. The periosteal cells exhibit a bluish haze but the intensity or rapid appearance of color seen in osteoblasts is not found in this region. Later bluish granules can be found in marrow

cells and, later still, a few small granules can be detected in osteocytes, that is, bone corpuscles. During the course of the observation, however, no change of color can be detected in the cells or intercellular matrix material of the cartilage. Fat globules present in the marrow display a reddish-purple tint and this color, which is quite unlike the deep indophenol blue, is also seen in some of the immature cartilage cells. The exact chemical reaction in the lipoid has not been pointedly studied save to say that it is not elicited by either of the two reagents alone.

The development of any blue color in the slice is easily prevented by cyanide. Such inhibition is not an absolute phenomenon since, as Friedenwald and Stiehler (19) have pointed out, a positive reaction may develop after a lag and may be prevented from developing further by the addition of more cyanide. If ferricyanide is added to the washed slice at the completion of the reaction, a faint bluish tint appears in the cartilage as well as the bony trabeculae indicating that the reagents have penetrated these tissues.

The significance of the presence of cytochrome oxidase in the osteoblast is not clear at present nor can its possible relation to osteoid formation or bone salt deposition be even hazarded. It is interesting that, in contrast, no activity is found in cartilage. Our observations have confirmed and amplified those of others (5, 35) in this latter respect.

Succinic Dehydrogenase: An histochemical method for the demonstration of succinic dehydrogenase has been reported by Semenoff (58) who applied the well known Thunberg technique to tissue sections. We have utilized this method, with certain controls, in studying free hand slices of cartilage and bone from rats and guinea pigs.

Slices are placed in a solution of Krebs-Ringer phosphate (pH 7.4) solution containing methylene blue (1–2000). They are allowed to remain in this medium 3 or 4 minutes or longer, removed, gently blotted and placed in a small pool of .1 M sodium succinate ringed by vaseline which has been extruded from a syringe through a large bore hypodermic needle. The fluid is then covered by a cover slip which is gently pressed onto the vaseline to produce an air-tight seal, care being taken not to entrap any air bubbles in the chamber thus formed. Two control preparations are set up: in one, Krebs-Ringer phosphate solution replaces the succinate substrate; while the second control slice is placed

in equal parts of .1 M sodium succinate and .1 M sodium malonate. All preparations are then incubated on a warm plate near the microscope.

Initially the entire section is colored blue, the nuclei more intensely than the cytoplasm of the cells and ground substance of the cartilage, which is usually metachromatic. The first change to take place is a fading of color in the cartilage so that a yellow band of tissue appears above the shaft and beneath the center of ossification. In preparations with just the proper degree of staining by methylene blue this decoloration precedes any loss of color in the underlying bone and marrow. We have not been able to detect any differences in the rate of decolorization of various regions of growing cartilage. Loss of color soon appears in the bone and marrow cells as well, but this change usually follows the decolorization of the cartilage. Control sections in the Krebs-Ringer phosphate medium ultimately become colorless, but at a slower rate; while those exposed to malonate show no decolorization. As a further control, the cover slip is removed, the transformation of the colorless leucomethylene blue to methylene blue may be observed as the tissue regains its original hue.

There have been several reports on the dehydrogenase activity of cartilage. Kuwabara (35) was apparently the first to apply the Thunberg technique to this tissue in the test tube. More specifically, Rosenthal et al. (52) have demonstrated succinic dehydrogenase activity in bovine articular cartilage. The histochemical studies reported above would confirm these findings although they are disappointing in that they fail to show any differences in enzyme activity in various regions of the cartilage.

Citric Acid Dehydrogenase: The importance of citric acid in the metabolism of bone stems from several lines of investigation. The effects of citrate on the healing of rickets was noted a number of years ago (61); at the present time the exact mechanism of this phenomenon is not clear but it appears as though more is concerned than a mere increase in the absorption of calcium from the intestinal tract as a result of the presence of citrate. The presence of rather large quantities of citric acid in bone and to a lesser extent in cartilage was demonstrated by Dickens (9). It seemed therefore of interest to determine whether cartilage displayed any dehydrogenase activity when placed in a substrate of citrate.

A technique similar to that described above for succinic dehydrogenase has been applied to study citric acid dehydrogenase activity. The only difference is the use of a substrate consisting of .1 M sodium citrate. Free hand sections are placed in this medium with methylene blue buffered at pH 7.4 and observed anaerobically. There is a fairly prompt disappearance of color from the cartilage before any color change is found in control slices observed in methylene blue-buffer mixture alone. It is thus apparent that cartilage contains a system capable of dehydrogenating citric acid.

Phosphatase: Since Robison's (49) demonstration of alkaline phosphatase in bone 25 years ago, this enzyme has been the focus of a storm of controversy over its relationship to the mechanism of bone formation. Robison (50) advanced the hypothesis that the deposition of bone salt in the organic matrix of cartilage involves the hydrolysis of an organic phosphate ester by phosphatase, thus leading to a precipitation of calcium phosphate in the tissue. The well known observations that serum and bone phosphatase values are elevated when there is an acceleration of new bone formation and depressed when osteoblastic activity is reduced, as in scurvy (24, 62) would make it seem likely that this enzyme has an important role in the calcification mechanism of bone and cartilage.

The development of an histochemical technique for the demonstration of alkaline phosphatase by Gomori (22) and Takamatsu (63) in 1939 has proved a means whereby phosphatase can be more precisely localized in tissues. In embryonic bone of chick (46) or rat (32, 34) enzymatic activity is found in the undifferentiated mesenchymal cells which give rise to cartilage. In developing bone, periosteal cells, as well as osteoblasts, display a prominent phosphatase reaction (17, 34). In addition, there is histochemical evidence of enzymatic activity in the hypertrophic cartilage cells as well as in the matrix substance between them but not in cartilage matrix elsewhere (23, 39). In contrast, bone matrix exhibits no phosphatase activity, while, as already noted, the osteoblast and, to a lesser degree, the osteocyte give positive reactions.

Our own studies have confirmed these observations. We have had difficulty, however, in using the technique advocated by Gomori (23) because of the presence of bone and have therefore resorted to studying serial sections one of which is incubated in the presence of glycerol

phosphate. After a suitable interval both are stained with silver nitrate and then compared. In our opinion the phosphatase reaction applied to bone is not a particularly suitable method since the duration of incubation, amount of washing etc. change the end result. We have therefore turned to quantitative chemical analyses which yield more precise data (15).

GLYCOGEN

Since the demonstration of glycogen (*zoamyline*) in cartilage by Charles Rouget in 1859 (55), a great many histochemical observations of this material have been reported. With time there has grown a feeling that the presence of glycogen might have some bearing on the calcification process. For instance, in 1885 Marchand (41) called attention to the increase in size of the cartilage cells at the growing ends of bones and ascribed this hypertrophy to an accumulation of glycogen in the cells. In 1928 Hoffmann et al. (31) stated that "a striking relationship exists between the disappearance of glycogen in cartilage and its ossification." A few years later Harris (27) also suggested that the presence of glycogen might have some bearing on the calcification process, possibly being related to Robison's (50) phosphatase mechanism. More recently the presence of glycogen in cartilage cells has assumed further importance in regards to the phosphorylase mechanism of Gutman (25, 26). Several other recent studies (21, 32) have confirmed and somewhat amplified the previous investigations.

Harris (28) made the pointed statement that "osteoblasts and vascular bone contain no glycogen." However, Gendre (20) seems to have noted glycogen in osteoblasts and osteocytes although his descriptions are extremely brief and not very clear.

In our own studies we have utilized the Bauer-Feulgen (1) and McManus (43) techniques for the demonstration of glycogen. The fixation has been absolute alcohol or absolute alcohol-picric acid-formol as recommended by Rossman (54). If silver nitrate is to be used to demonstrate lime salts, the former should, of course, be employed. Glycogen was differentiated from other materials, particularly the ground substance of cartilage by the use of control sections exposed to saliva. The presence of glycogen in the cartilage cell has been confirmed. Glycogen is found in increasing amounts as the cartilage cell matures.

In young and most undifferentiated cells only a few small pink or reddish granules can be found. With multiplication of the cells and as they increase in size, more numerous and larger granules of glycogen appear, until, in the hypertrophic cells close to the cartilage-bone junction, the cytoplasm is filled with homogeneous reddish-staining material. No glycogen can be found in the cell nuclei at any time. The most mature cells, that is those closest to the shaft and nearest the invading blood vessels, do not appear to contain glycogen. It is this area that we have observed most carefully, realizing the importance which the disappearance of glycogen may have on current concepts (25, 26) of the calcification mechanism in cartilage. We have, therefore, studied sections stained for glycogen and similar ones exposed to silver nitrate in order to bring out the exact site of lime salt deposition (a pointed comparison which to our knowledge has not heretofore been carried out). From our observations it appears that there is a rough inverse relationship to the presence of glycogen and presence of lime salt deposition in the zone of provisional calcification. In cartilaginous matrix where positive silver staining is found little or no glycogen is present in the 2 or 3 adjacent cells. The next few cells above usually contain large quantities of glycogen together with a spotty deposition of silver in the adjacent matrix. For an absolute answer to this question it would seem desirable to apply quantitative chemical methods in order to determine the relationships between the concentrations of glycogen and calcium and phosphate. Such studies are now in progress (16). Small amounts of glycogen have been observed in osteoblasts and osteocytes.

MUCOPOLYSACCHARIDES

Many cells and tissues, among them cartilage, have long been known to exhibit metachromasia; that is, when stained wth a specific group of dyes, one of which is toluidine blue, they exhibit not the expected blue (orthochromatic) color of the dye but a different (metachromatic) tint. In the case of cartilage the matrix substance is reddish-purple (metachromatic). To offer an explanation for this phenomenon one has to consider the properties of both the tissue and the dye. In the case of the former, most cells and tissues exhibiting metachromasia contain polymerized esters of sulfuric acid and a carbohydrate (38). Dyes

exhibiting metachromasia are apparently polymerized, especially in concentrated solutions (44, 45). In such a state they change color; this, of course, may be demonstrated by measuring the absorption maxima at various wave lengths. The cells and tissues capable of exhibiting metachromasia seem to have the ability to adsorb the dye, hence increasing its polymerization; as a result a different color is observed in such areas, indicating the presence of mucopolysaccharide.

Cartilage, of course, contains large quantities of chondroitin sulfuric acid bound to protein. The former may be separated and gives rise to sulfuric acid and chondroitin, which latter moiety yields a galactosamine, glucuronic acid and acetic acid. As already noted, cartilage, at least the matrix substance and cytoplasm of hypertrophic cells, is extremely metachromatic. This property has been the subject of several studies of which those from Wislocki's laboratory (7, 8, 64) are most interesting. These investigators have extended observations on the well known metachromatic properties of cartilage by observing the effect of varying the hydrogen ion concentration on the staining of cartilage sections by methylene blue or toluidine blue. It was found that in acid media (pH 1.5–3.0) only the cartilage cell capsules exhibited any metachromatic staining; as the environment became less acid (pH 5.0–7.0) the matrix also took on this staining property. The Boston group has further noted that cartilage matrix gives a positive color with the Bauer stain a property which is not destroyed by saliva (64) In addition, metachromasia of cartilage matrix of fixed tissue is not destroyed by hyaluronidase derived from testis (8) in the concentrations used.

We have repeated and confirmed these observations, employing similar techniques and have included the periodic acid method of McManus (43) which has been shown to be a specific method for demonstrating certain polysaccharides (33). Our observations have lead to little that is new save for one extremely interesting finding: osteoid, like cartilage matrix, is metachromatic. We have noted very thin metachromatic bands along the inner margin of the cortex in undecalcified sections. That such bands are osteoid has been amply confirmed in studies on rachitic animals which will be reported elsewhere (16). Another observation of probable significance is the prominent metachromasia of the fibrillar structure of cartilage and bone

matrix. In suitably stained preparations there is a definite concentration of dye along these fibrils with lighter zones in between.

NUCLEOPROTEINS

Little information is available on the nucleoproteins of cartilage and bone. Chemical analyses of the nucleoprotein phosphorus of embryo and adult sheep cartilage have revealed definite though small amounts; greater quantities are found the younger the tissue (6). The concentrations of nucleoprotein in cartilage appear to be among the lowest of any tissues thus far examined. In order to obtain further information we have applied certain histochemical procedures to undecalcified sections of cartilage and bone in order to differentiate the two types of nucleoprotein, those containing desoxyribose nucleic acid and those containing ribose nucleic acid.

Desoxyribose nucleic acid: For the demonstration of desoxyribose (thymus) nucleic acid the Feulgen (12) reaction is customarily employed. As is well known, this technique consists of hydrolysis of the nucleo-protein with warm normal hydrochloric acid in order to free the aldehyde group of the desoxyribose sugar which is then identified by the Schiff reagent.

When this procedure is applied to undecalcified sections of cartilage and bone only the nuclei of cells (cartilage, bone and marrow) stain. There is no coloration of the cytoplasm of cartilage cells or matrix of cartilage and bone. The identity of the Feulgen-positive material has been further confirmed by incubation of the section in the presence of gelatin and magnesium sulfate in Veronal buffer (42) with a desoxyribonuclease prepared from calf thymus and kindly furnished by Dr. Norman Weissman. Following such treatment the Feulgen positive areas noted above fail to stain.

Ribose nucleic acid: Since the initial observation of Brachet (4) in 1940 a number of experiments have been carried out utilizing the effect of ribonuclease to destroy the affinity of cells for basic dyes such as methylene blue or hematoxylin.

We have used formalin fixed or frozen dried tissue stained with methylene blue or hematoxylin. Some of the sections were incubated with a ribose nuclease prepared from pancreas and kindly supplied by Dr. Norman Weissman. Sections were incubated with this prepara-

tion in acetate buffer at pH 5.0. No effect was found on the basophilia in the cartilage matrix; this confirms observations reported by Dempsey and Singer (8) in tracheal cartilage.

LIPOID

Neutral Fat: Leydig (37) was apparently one of the first to mention and illustrate (page 33, Fig. 16B) fat droplets in cartilage cells. A number of observers (2, 57, 59) have studied the distribution of neutral fat in the cartilage cells of various animals. There is agreement that the presence of sudanophilic material is not evidence for any "degenerative" change in the cartilage cell since one can follow a definite cycle in the appearance, accumulation and disappearance of fat; this coincides with the growth and maturation of the cartilage cell. The fat, however, disappears as the stage of hypertrophy of the cells is reached.

Our own observations have confirmed those already reported in the literature. Fat globules stained with Sudan IV are absent from perichondrial cells; as these most immature cells become larger, small sudanophilic droplets in the cytoplasm coalesce to form larger ones. In the proliferating cartilage just after the formation of rows, fat reaches its greatest prominence. As the cells increase in size and approach the zone of provisional calcification fat droplets have completely disappeared from the cells. Although the simultaneous identification of fat and glycogen in our material has not been possible one gets the impression that the former precedes the latter in maximum accumulation in the cytoplasm as the cell matures.

HYDROGEN ION CONCENTRATION

Not a great deal is known concerning the hydrogen ion concentration of cartilage and bone, although local changes are often invoked to explain the calcification mechanism. One of the earliest series of observations was performed by Rous (56) who injected litmus as well as other dyes into animals. The following changes were found: *litmus* (red at pH 6.2, blue at pH 8.4), cartilage matrix "sky-blue", while bone was deeper blue; bromocresyl green (yellow at pH 4.0, blue at pH 6.0), cartilage, blue; chlorphenyl red (yellow at 5.3, purple at pH 6.2), cartilage and bone, rose purple; bromocresyl purple (yellow at pH 5.4, blue at pH 7.0), cartilage matrix, purplish blue, bone, deep

blue; cresol red (yellow at pH 7.2, red at pH 8.8), cartilage, yellow. Dempsey et al. (7) have noted that cartilage gives a red-orange color with neutral red (red at pH 6.8, yellow at pH 8.0). Pierce (48) used the guinhydrone electrode to measure the pH of normal resting and proliferating cartilage cells and found values of 7.35 and 7.39 respectively.

We have applied a series of indicator dyes to fresh slices of cartilage and bone in order to determine whether any information could be obtained as to the approximate pH of the tissue. The results are summarized in Table 1. It will be seen from this that cartilage has an over-

TABLE I

INDICATOR	pH RANGE	COLOR CHANGE	COLOR OF CARTILAGE
Bromphenol blue	3.0–4.6	Yellow-blue	Blue
Bromcresyl green	4.0–5.6	Yellow-blue	Blue
Methyl red	4.2–6.3	Red-yellow	Yellow
Litmus	4.5–8.3	Red-blue	Blue
Chlorphenyl red	5.0–6.6	Yellow-red	Red
Bromcresyl purple	5.4–7.0	Yellow-purple	Purple
Bromthymol blue	6.0–7.6	Yellow-blue	Blue
Phenol red	6.6–8.2	Yellow-red	Orange
Neutral red	6.8–8.0	Red-yellow	Orange
Cresyl red	7.2–8.8	Yellow-red	Yellow
Phenolpthalein	8.3–10.0	Colorless-red	Colorless

all pH in the range of pH 7.4. Perhaps the most significant finding is a negative one—that the pH of cartilage is not alkaline.

We have also applied a series of oxidation-reduction indicators of known potential to fresh slices of cartilage and bone in an attempt to detect differences in potential. The results have been difficult to interpret and will not be commented upon at this time.

DISCUSSION

There are certain important aspects of the metabolism of cartilage and bone with relation to growth processes which have been reported but have not been mentioned in what has gone before. It would seem appropriate to call attention to these, point out certain integrations and suggest further directions for study.

It is apparent that a beginning has been made in our understanding of the biochemistry of cartilage. A number of observations, many admittedly unrelated, have been recorded. The problem has been approached from several standpoints: interest in exploring the metabolism of a virtually avascular tissue such as cartilage, an attempt to obtain information which might throw some light on the arthritis problem, and the hope of elucidating the calcification mechanism.

At the outset it should be pointed out that a variety of animals have been used, that the age of the animals have varied from group to group and that articular cartilage, which would appear to be a most sluggish tissue, has been most commonly employed in the studies reported. However, it has been shown that cartilage has a definite, though low QO_2 (10, 30, 40, 51); this is related to the age of the animal (53) and more important to the site from which the specimen for study is taken, rate of growth playing an important role (10, 53). In addition it has been found that cartilage has aerobic and anaerobic glycolysis (5, 11, 35, 51), the latter being more marked. Methylene blue has a markedly stimulating effect on metabolism, increasing the QO_2 of cartilage many times (5, 30, 52). This observation has led to a study of dehydrogenase systems in cartilage and it would appear that enzymes capable of dehydrogenating glucose (52), mannose (52), succinate (52), lactate (52) and pyruvate (52) are present. In addition, the presence of glycogen has, of course, aroused a great deal of interest; this phase will be dealt with shortly. Glycogen was early shown to break down into lactic acid (31). Cartilage appears to metabolize certain hexose-phosphate compounds (40). The possible role of these with especial reference to the presence of a phosphorylase (25, 26) which would aid in the local mobilization of phosphate is doubtless of importance. The demonstration of a citric acid dehydrogenase in cartilage referred to above may also be of some significance because of the presence of this material in cartilage (9) and its effect on the healing of rickets (61). Phosphatase which has been the center of such a controversial storm for many years will be mentioned more in detail below. Our failure to find cytochrome oxidase activity in cartilage confirms brief references to similar findings in the literature (5, 35). From the above observations one can hardly look upon cartilage as a metabolically inert material. To be sure articular cartilage is much more sluggish than

cartilage cells which are proliferating. It is hoped that more attention will be focused on the latter type of tissue with special emphasis on its metabolism with respect to bone formation.

When we consider the biochemistry of bone itself the void is virtually complete. Furthermore it would seem that there is little approach in the way of orthodox biochemical procedures due to the presence of marrow cells. Histochemical studies would seem to be extremely important here. The presence of cytochrome oxidase in osteoblasts has been referred to, though its significance in these cells is not at all clear. Phosphatase activity can also be visualized in osteoblasts in which cells glycogen is also present. The organic portions of bone may be studied histochemically as well, at least to demonstrate polysaccharide moieties (29, 64). An approach to the biochemistry of the osteoblast might be made through the use of periosteal cells which can be stripped from the bone and thus provide a more or less pure culture of osteoid forming material. Such an approach has already provided quantitative data regarding phosphatase activity of periosteum (15).

At the present time certain points have been established regarding the possible mechanism of the calcification process in cartilage matrix. The dynamics of the phenomenon in osteoid (bone matrix) is less clear.

From the data which are available it seems fairly clear that both humoral and local concentrations of inorganic materials are extremely important. This role of the former in the pathogenesis of rickets need only be mentioned since the prompt deposition of lime salts in the intact organism as well as in cartilage slices *in vitro* (60) is too familiar to warrant further discussion. Local factors responsible for the deposition of inorganic salts have been concerned with mechanisms for increasing phosphate concentration. Little recent attention has been paid to the deposition of calcium or carbonate, save that the possible role of carbonic anhydrase in the latter's appearance has been suggested (3). The story of phosphate deposition began with the demonstration of the enzyme alkaline phosphatase by Robison (49). This led to a tremendous amount of work on this enzyme and possible substrates for it, culminating in the conjecture by Harris (49) that glycogen might be related in some way to the phosphatase mechanism. With the gradual evolution of our knowledge of the glycogenolytic cycle such a con-

cept seems even more plausible, particularly too, since at least one enzyme, phosphorylase, which changes glycogen to hexosephosphate has been identified by Gutman and his co-workers (25, 26) in epiphyseal cartilage. No other enzymes concerned in the breakdown of glycogen have as yet been identified nor, of course, has phosphorylase activity been precisely localized at the site of glycogen breakdown. Currently, then, glycogen is thought to be the beginning material for a series of reactions which would yield a phosphate ester from which phosphatase would liberate free phosphate. It is of further interest that one of us, in association with Dr. M. D. Levine (36) has demonstrated the presence of a lecithinase in cartilage which will break down lecithin into a diglyceride and phosphocholine; the latter is assumed to be broken down by alkaline phosphatase. It would seem then that there are at least two unrelated mechanisms for providing an increased concentration of phosphate in the zone of provisional calcification of cartilage.

Little save conjecture is known of the local or tissue factors which must also be important in the calcification mechanism. The possible relationship of matrix to calcium deposition was first mentioned 40 years ago by Pfaundler (47) and later studied by Freudenberg and György (18). It has seemed of interest to us that bone matrix and cartilage matrix exhibit the same staining reactions with the metachromatic dye, toluidine blue and the periodic acid-leuco fuchsin reaction. This may indicate that chemically these two structures are closely related.

The basophilia of cartilage is doubtless due to the presence of chondroitin-sulfuric acid. We have not as yet carried out any experiments designed to abolish this basophilia save to demonstrate that it is not destroyed by two types of nucleoproteinases.

SUMMARY

A study has been carried out on normal cartilage and bone applying certain histochemical techniques in an attempt to gain a base from which to study certain diseases. The results of these techniques are presented and discussed.

BIBLIOGRAPHY

1. Bauer, H., Z. micro. anat. Forsch., **33:** 143, 1933.
2. Bell, E. T., Am. J. Anat., **9:** 401, 1909.

3. Benesch, R., Chance, M. R. A., and Glynn, L. E., Nature, **155:** 203, 1945
4. Brachet, J., C. R. Soc. Biol., **133:** 88, 1940.
5. Bywaters, E. G. L., J. Path. and Bact., **44:** 247, 1937.
6. Davidson, J. N., and Waymouth, C., Biochem. J., **38:** 39, 1944.
7. Dempsey, E. W., Bunting, H., Singer, M., and Wislocki, G. B., Anat. Rec., **98:** 417, 1947
8. Dempsey, E. W. and Singer, M., Endocrinol., **38:** 270, 1946.
9. Dickens, F., Biochem. J., **35:** 1011, 1941.
10. Dickens, F., and Weil-Malherbe, H., Nature, **138:** 125, 1936.
11. Ehrlich, P., Das Sauerstoff-Bedurfniss des Organisms, A. Hirschwald, Berlin, 1885.
12. Feulgen, R., and Voit, K., Arch. ges. Physiol., **206:** 389, 1924.
13. Flexner, L. B., J. Biol. Chem., **131:** 703, 1939.
14. Follis, R. H., Jr., and Berthrong, M., Am. J. Path., **24:** 685, 1948.
15. Follis, R. H., Jr., Fed. Proc., **8:** 480, 1949.
16. Follis, R. H., Jr., Unpublished observations.
17. Freeman, S., and McLean, F. C., Arch. Path., **32:** 387, 1941.
18. Freudenberg, E., and György, P., Biochem. Zeit., **110:** 299, 1920.
19. Friedenwald, J. S., and Stiehler, R. D., Arch. Ophth., **20:** 761, 1938.
20. Gendre, H., Bull. Histol. Tech. Micro., **15:** 165, 1938.
21. Glock, G. E., J. Physiol., **98:** 1, 1940.
22. Gomori, G., Proc. Soc. Exp. Biol. and Med., **42:** 23, 1939.
23. Gomori, G., Am. J. Path., **19:** 197, 1943.
24. Gould, B. S., and Shwachman, H., Am. J. Physiol. **135:** 485, 1942.
25. Gutman, A. B., and Gutman, E. B., Proc. Soc. Exp. Biol. and Med., **48:** 87, 1941.
26. Gutman, A. B., Warrick, F. B., and Gutman, E. B., Science, **95:** 461, 1942.
27. Harris, H. A., Nature, **130:** 996, 1932.
28. Harris, H. A., Bone Growth in Health and Disease, London, 1933.
29. Hempelmann, L. H., Jr., Anat. Rec., **78:** 197, 1940.
30. Hills G. M., Biochem. J., **34:** 1070, 1940.
31. Hoffmann, A., Lehmann, G., and Wertheimer, E., Pfluger's Archiv., **220:** 183, 1928.
32. Horowitz, N. H., J. Dent. Res., **21:** 519, 1942.
33. Hotchkiss, R. D., Arch. Biochem., **16:** 131, 1948.
34. Kabat, E. A., and Furth, J., Am. J. Path., **17:** 303, 1941.
35. Kuwabara, G., J. Biochem. (Jap.), **16:** 389, 1932.
36. Levine, M. D., and Follis, R. H., Jr., Fed. Proc., **8:** 458, 1949.
37. Leydig, F., Lehrbuch der Histologie des Mensche und der Thiere, Frankfurt, 1857.
38. Lison, L., Histochimie Animale, Paris, 1936.
39. Lorch, I. J., Quart. J. Micro. Sc., **88:** 367, 1947.
40. Lutwak-Mann, C., Biochem. J., **34:** 517, 1940.
41. Marchand, F., Virch. Arch., **100:** 42, 1885.
42. McCarty, M., J. Gen. Physiol., **29:** 123, 1946.

43. McManus, J. F. A., Nature, **158:** 202, 1946.
44. Michaelis, L., and Granick, S., J. Am. Chem. Soc., **67:** 1212, 1945.
45. Michaelis, L., Cold Spring Harbor Symposia on Quantitative Biology, **12:** 131, 1947.
46. Moog, F. B., Biol. Bull., **86:** 51, 1944.
47. Pfaundler, M., Jb. Kinderheilkund., **60:** 123, 1904.
48. Pierce, J. A., J. Biol. Chem., **124:** 115, 1938.
49. Robison, R., Biochem. J., **17:** 286, 1923.
50. Robison, R., and Soames, K. M., Biochem. J., **18:** 740, 1924.
51. Rosenthal, O., Bowie, M. A., and Wagoner, G., J. Cell. and Comp. Physiol., **17:** 221, 1941.
52. Rosenthal, O., Bowie, M. A., and Wagoner, G., J. Cell. and Comp. Physiol., **19:** 15, 1942.
53. Rosenthal, O., Bowie, M. A., and Wagoner, G., J. Cell. and Comp. Physiol., **19:** 333, 1942.
54. Rossman, I., Am. J. Anat., **60:** 270, 1940.
55. Rouget, C., J. de la Physiol., **2:** 308, 1859.
56. Rous, P., J. Exp. Med., **41:** 379, 739, 1925.
57. Sacerdotti, C., Virch. Arch., **159:** 152, 1900.
58. Semenoff, W. E., Zeit. f. Zellforsch. u. Micro. Anat., **22:** 305, 1935.
59. Sheehan, J. F., J. Morph., **82:** 151, 1948.
60. Shipley, P. G., Bull. Johns Hopkins Hosp., **35:** 304, 1924.
61. Shohl, A. T., J. Nutrition, **14:** 69, 1937.
62. Shwachman, H., and Gould, B. S., J. Nutr., **23:** 271, 1942.
63. Takamatsu, H., Trans. Jap. Path. Soc., **29:** 492, 1939.
64. Wislocki, G. B., Bunting, H., and Dempsey, E. W., Am. J. Anat., **81:** 1, 1947.

PLATE 1

Explanation of Figures

Figure 1. Free hand slice of rat bone treated with the nadi reagent. There are accumulations of pigment about osteoblasts whose nuclei do not stain.

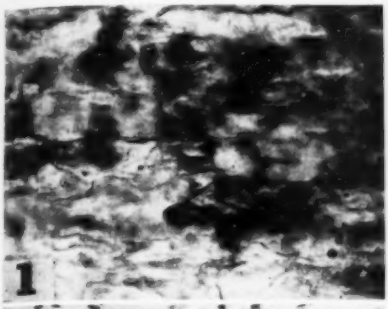
Figure 2. Higher power of free hand slice similar to Figure 1.

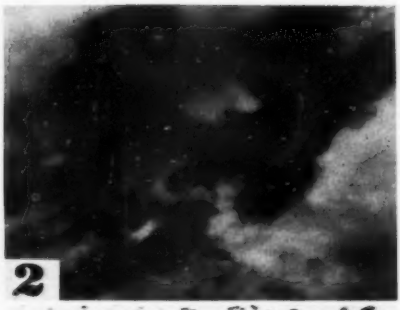
Figure 3. Section of undecalcified normal rat rib stained with 2% silver nitrate.

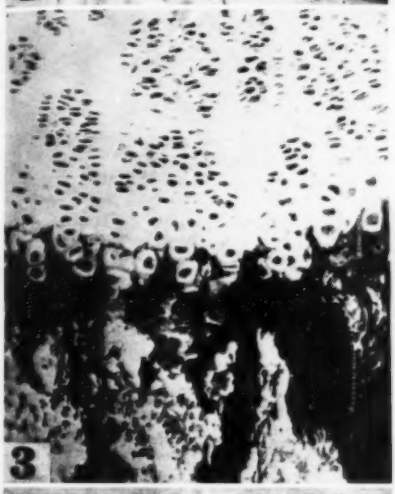
Figure 4. Similar section incubated with buffered sodium glycerophosphate for 2 hours and then stained with silver nitrate. Note increase in silver staining in cartilage matrix and hypertrophic cells. The marrow cells also give a positive reaction.

Figure 5. Section stained with the periodic acid-leucofuchsin technique. The dark staining material in the hypertrophic cartilage cells is glycogen.

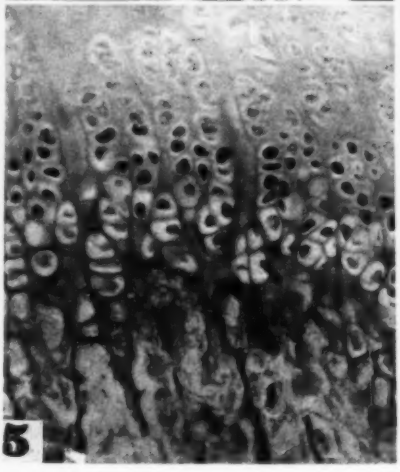
Figure 6. Frozen section of human costal cartilage stained with Sudan IV to demonstrate fat globules which stain more darkly than the cell nuclei.

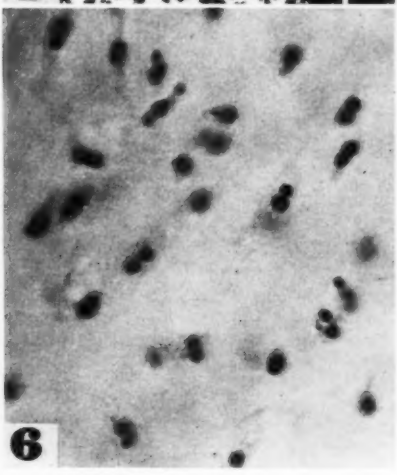

1


2


3

4


5


6

ACUTE HEPATITIS IN GENERAL HOSPITAL PRACTICE[1]

The Rôle of Transfusions and Inoculations

OSCAR D. RATNOFF and GEORGE S. MIRICK

From the Department of Medicine, The Johns Hopkins University School of Medicine

Received for publication July 6, 1949

The transfusion of whole blood or plasma has been employed with increasing frequency in the treatment of a wide variety of disease states. At the same time, impressive evidence has accumulated that this procedure is not without risk. Among these risks, the appearance of hepatitis some time after the transfusion of blood has been described repeatedly in recent years. This syndrome, usually called homologous serum jaundice, has been observed after the injection of whole blood, plasma or serum, and after the use of needles and syringes contaminated with unsterilized blood or serum. During the last few years, the number of blood and plasma transfusions given at The Johns Hopkins Hospital has risen sharply. It seemed of interest, therefore, to try to estimate the extent of the problem presented by homologous serum jaundice in hospital practice. The data to be presented emphasize the fact that, at present, homologous serum jaundice and acute infectious hepatitis cannot be distinguished on clinical grounds.

(*1*) *Description of the Case Material:* The histories were reviewed of all cases of hepatitis due to any cause in patients who were hospitalized at The Johns Hopkins Hospital between January 1, 1937 and December 31, 1948. Those patients with hemolytic or obstructive jaundice, amebic hepatitis, Weil's disease, hepatitis due to sulfonamides or cinchophen, or cirrhosis of the liver were eliminated from consideration. There remained 287 patients with acute infectious hepatitis (or catarrhal jaundice), acute yellow atrophy of the liver, post-arsphenamine hepatitis, and homologous serum jaundice. (Table I).

Which cases one should call homologous serum jaundice cannot be determined with certainty. Different authors give different estimates for the incubation period of this disease. These vary from about 2

[1] These studies were conducted under contract with the office of Naval Research, U. S. Navy.

weeks to 7 months (1–4). In the analysis which follows, any instance of hepatitis is called homologous serum jaundice if it appeared between 5 weeks and 5 months after the injection of human blood or its products. It is obvious that naturally occurring acute infectious hepatitis may have appeared in some patients following transfusion, and it is therefore impossible in any given instance to be certain of the route of infection.

Moreover, a review of the clinical and pathologic features of the cases of hepatitis studied demonstrated that they could not be satisfactorily differentiated. Although the cases designated as homologous

TABLE I

The Relative Frequency and Mortality of Various Types of Hepatitis

TYPE OF CASE	NO.	TOTAL	NO. FATAL CASES	CASE FATALITY
		%		%
Hepatitis after Injection of Homologous Blood or Blood Product	40	14.0	11	27.5
Hepatitis after Injection of Arsenical Compounds*	51	17.8	6	11.8
Hepatitis after Insertion of Other Needles†	39	13.6	2	5.1
Hepatitis during Pregnancy	22	7.7	2	9.1
All other Acute Hepatitis	148	51.5	12	8.1
Total Cases, Excluding Duplications	287		32	11.1

* Including 10 non-fatal cases treated with arsenicals during pregnancy.

† Including 2 non-fatal and 1 fatal case after insertion of needles during pregnancy.

serum jaundice were more severe than the average, no other essential difference was noted. In the group considered, regardless of the designation of the illness, most patients presented a relatively benign picture of abdominal complaints followed after several days by jaundice, light colored stools, and dark urine. Examination in the hospital disclosed that all the patients were jaundiced. Frequently the liver was palpable, and occasionally the spleen was as well. Ordinarily the process gradually subsided. Occasionally, however the hepatitis was more severe, and 32 patients, 11.2 per cent of the total, died. The high mortality rate may have been due in part to the fact that only the more obviously ill patients with hepatitis were admitted to the hospital during most of the period of this study.

Acute hepatitis was observed in 22 pregnant women. There was no

particular relationship between the time of onset of the hepatitis, its severity, and the stage of pregnancy. Five of these patients aborted during their illness, including 2 patients who died and 2 others who were under treatment for syphilis with arsenical compounds. Five other patients had premature deliveries during the course of their illness.

TABLE II

Laboratory Studies in Patients with Hepatitis

DIAGNOSIS		WBC CELLS PER MM³			SR MM/HR.			ALK. PHOSPHATASE (B.U.)		C.F.		T.T.		
		<10,000	10-12,000	>12,000	<10	10-20	>20	0-12.0	>12.0	0 – +	++ – ++++	≤6	6-9.9	10+
Hepatitis after Injection of Homologous Blood or Blood Product	Non-fatal cases..	19	6	3	4	3	5	19	6	7	10	2	0	11
	Fatal cases.....	5	2	4	0	1	0	5	1	0	6	0	0	3
Hepatitis after Insertion of Other Needles														
Arsenical	Non-fatal cases..	36*	2	6†	6	14	13*	10‡	10§	2	4	1	0	0
	Fatal cases.....	2	2	2	0	1	1	1	1	0	0	0	0	0
Non-Arsenical	Non-fatal cases..	28‖	4	2	5	7	8	20	3	4	8	1	1	9
	Fatal cases.....	1‖	0	1	0	0	2‖	1‖	0	0	0	0	0	0
Hepatitis During Pregnancy (total)	Non-fatal cases..	10	1	7	0	0	3	6	2	1	0	0	0	1
	Fatal cases.....	1	0	1	0	0	1	1	0	0	0	0	0	0
All other Acute Hepatitis	Non-fatal cases..	110	10	13	12	41	40	59	11	5	33	1	1	19
	Fatal cases.....	6	1	5	3	1	1	3	1	0	2	0	0	2
Total Excluding Duplications	Non-fatal cases..	196	23	28	27	65	62	110	30	19	55	5	2	40
	Fatal cases.....	14	5	13	3	3	4	10	3	0	8	0	0	5

* Including 6 pregnant women.
† Including 3 pregnant women.
‡ Including 4 pregnant women.
§ Including 2 pregnant women.
‖ Including 1 pregnant woman.

In one of these 5 cases, the baby died 8 hours after birth; there was no evidence of hepatitis in the fetal liver. The case fatality rate of hepatitis was the same in pregnant as in non-pregnant patients.

Laboratory studies did not help to differentiate the patients with acute hepatitis (Table II). In this series, bilirubinemia was invariably present. The sedimentation rate measured by the Wintrobe method was elevated above 20 mm. per hour in 41 per cent of the patients

tested, and was no more frequently elevated in fatal than in non-fatal cases. The white blood cell count was less than 10,000 per mm^3 in 75.1 per cent of the patients with acute hepatitis, between 10,000 and 12,000 in 10.0 per cent, and above 12,000 in 14.7 per cent. In many of these patients, extraneous factors may have been responsible for the leukocytosis. Leukocytosis was present more frequently in pregnant patients and in patients who died. The alkaline phosphatase activity of the serum was 12 Bodansky units or less in 120 of 153 patients in whom it was determined. It was not elevated above 12 Bodansky units any more frequently in fatal than in non-fatal cases. However, it is noteworthy that the alkaline phosphatase activity of the serum was more than 12 Bodansky units in 11 of 22 patients with post-arsphenamine jaundice, although none of these patients had erythema of the ninth day (5). The cephalin cholesterol flocculation test was positive, 2 plus or higher, in 63 of 82 patients, including all of 8 patients who died. The thymol turbidity was 10 Maclagan units or higher in 45 of 52 patients tested, and between 6 and 10 units in 2 of the others. In each of the 5 fatal cases tested, the thymol turbidity was higher than 10 units.

The liver was examined at autopsy in 28 of 32 fatal cases, and by biopsy in 7 non-fatal cases. No features were described which distinguished between acute infectious hepatitis, homologous serum jaundice, or post-arsphenamine hepatitis. When the destruction of the hepatic parenchyma was particularly severe, the diagnosis of acute yellow atrophy was often made, but the etiology of the lesion could not be determined pathologically.

(*2*) *The Incidence of Previous Injections of Human Blood in Patients with Acute Hepatitis:* During the period of this study, 40 patients were hospitalized with hepatitis in whom the first symptom appeared 5 weeks to 5 months after the injection of human blood or a fraction of human blood. Twenty-eight of these 40 patients had been injected with whole blood, 8 with whole blood and plasma, 2 with plasma alone, one with yellow fever vaccine from a known icterogenic lot (6), and one with human globulin. In other words, one out of every 7 patients hospitalized with hepatitis had been injected with human blood or a blood product 5 weeks to 5 months before the onset of the hepatitis. Only 20 of these patients were admitted during the first 10 years covered by this study, whereas an additional 20 were hospitalized

during the last 2 years. This increased incidence may reflect the more common therapeutic use of blood and its products in the last few years, since the number of patients with hepatitis of all types admitted annually throughout the study remained approximately constant.

The hepatitis which followed injection of human blood was often severe, and 11, 27.5 per cent of the 40 patients with this history, died. On the other hand, only 21, or 8.5 per cent of the remaining patients, died.

(*3*) *The Incidence of Parenteral Injections Other than Blood in Patients With Acute Hepatitis:* The transmission of hepatitis through the use of contaminated syringes and needles has been described repeatedly (7–13). There were 247 patients with acute hepatitis who had not previously had an injection of human blood. Ninety of these patients were known to have had a parenteral test or parenteral injection 5 weeks to 5 months before the onset of their disease. The nature of the histories reviewed was such that it is likely that more of the cases of hepatitis may have followed parenteral inoculation. Fifty-one of the 90 patients were under treatment with arsphenamine, neoarsphenamine, tryparsamide, or mapharsen for syphilis. Arsenic is a known hepatotoxic agent, and some or all of the patients under treatment with arsenical compounds may have had a true arsenical hepatitis. However, it is of note that 13 of these 51 patients were subsequently treated with mapharsen without a single instance of recurrent hepatitis. There remained 39 patients whose hepatitis followed 5 weeks to 5 months after a parenteral test or injection other than arsenic.

The meaning of these data depends upon a knowledge of the frequency of a history of parenteral tests or injections in patients hospitalized for causes other than hepatitis. For this reason, an estimate was made of how frequently control patients without hepatitis gave a history of parenteral tests or injections before they became ill. The case histories of 287 patients without hepatitis, admitted to The Johns Hopkins Hospital during the period of this study, were reviewed. In each instance the control patient was the one whose admission directly followed on the same ward that of a patient with hepatitis. In this manner, an equal number of controls was assembled, similar to the patients with hepatitis in respect to age, sex, race, economic status and time of admission to the hospital. Each control chart was searched for

a history of a parenteral test or injection 5 weeks to 5 months before the onset of the illness for which the patient was hospitalized. In the analysis which follows, the cases of pregnant women were eliminated from consideration in both the hepatitis and control series, since these cases were not comparable in the 2 groups. In the control series, the pregnant patients were usually admitted to the hospital in labor and had had blood studies during their pregnancy. Most of the patients with hepatitis, on the other hand, were hospitalized early during the course of their pregnancy, before any blood studies had been performed.

There remained 264 patients with hepatitis and 268 control patients. In the period between 5 weeks and 5 months before the onset of the illness for which they were hospitalized, 2 of the control patients had been transfused, 8 had been treated with arsenical compounds, and 20 of the remaining 258 patients had had a parenteral test or injection. On the other hand, 40 of the patients with hepatitis had been transfused, 41 had been injected with arsenical compounds, and 36 of the remaining 183 patients had had a parenteral test or injection. In other words, only 8 per cent of the control patients had previously had a parenteral test or injection of material other than blood or arsenic, compared with 20 per cent of the patients with hepatitis. The probability that this difference could have occurred by chance is less than one in 100. The data, therefore, suggest that there may possibly have been a causal relationship between the preceding parenteral test or injection and the hepatitis.

(*4*) *The Incidence of a Previous Injection of Human Blood in Patients who Died of Acute Hepatitis:* During the period of study, 32 patients with acute hepatitis, postarsphenamine hepatitis, acute yellow atrophy of the liver, or homologous serum jaundice died. Eleven of these 32 patients had had an injection of human blood, plasma, or globulin before the onset of their hepatitis.

(*5*) *The Incidence of Hepatitis in Patients Transfused with Blood or Plasma:* The available data do not answer the question of how frequent hepatitis was in relation to the number of blood transfusions given. Only sporadic information was available concerning the number of transfusions of blood and plasma given at the hospital before 1946. In this study, no attempt has been made to follow each patient who had been transfused to learn whether he subsequently had hepati-

tis. Only those who were *hospitalized* because of their hepatitis were included in the series. It has been ascertained that other patients, not included in this series but transfused at The Johns Hopkins Hospital, have been under treatment elsewhere for acute hepatitis (14). On the other hand, patients transfused elsewhere who came to The Johns Hopkins Hospital with hepatitis were included in the present series. Finally, it may be assumed that only the more obviously ill patients were admitted to the hospital. The invariable presence of jaundice in the patients hospitalized supports this view.

Approximately 2800 patients per year were transfused at The Johns Hopkins Hospital with blood, plasma, or both, in 1947 and 1948. These figures include 2100 patients who were transfused with blood, and 1000 patients with plasma. During this same period, approximately 8.5 patients per year were hospitalized with hepatitis after blood or plasma transfusions which had been given at this hospital, and 2 per year died. The proportion of patients re-admitted to The Johns Hopkins Hospital with hepatitis after transfusion, then, was one to each 330 patients transfused; and the proportion of fatalities was one to each 1400 patients transfused. For the reasons outlined, the actual incidence of the disease was undoubtedly higher. The relative incidence of hepatitis after transfusions of blood compared with plasma could not be determined since some of the patients received both.

DISCUSSION

Except for the use of human volunteers, no method is available at present to study the etiology of acute infectious hepatitis. For this reason it is not possible to state with certainty in any particular case that a causal relationship exists between the injection into a patient of human blood and the subsequent development of acute hepatitis. None the less, it is impressive that 14.0 per cent of the patients admitted to The Johns Hopkins Hospital with acute hepatitis had been transfused between 5 weeks and 5 months before. This period of time is the same as the incubation period previously reported when hepatitis followed the injection of homologous blood in human subjects (1–4). Tentatively, then, these patients may be presumed to have had homologous serum jaundice.

Hepatitis after transfusion, as it was seen in the hospital, was a

serious and often fatal disease. In the present series, 27.5 per cent of these patients died. This fatality rate is comparable to that reported by others. For example, Sheinberg, Kinney, and Janeway (15) observed that 4 of 11 patients with homologous serum jaundice after transfusion died. And Snell, Wood, and Meienberg (16) observed a case fatality rate of 19 per cent. The reported fatality rates may have been too high, since probably only the more severely ill patients were hospitalized. None the less, the disease appeared to be much more severe than the homologous serum jaundice which followed vaccination with yellow fever vaccine containing human serum (6). Very likely this difference was due to the much larger inoculum of virus which occurred during blood transfuson. It is noteworthy that the case fatality rate of hepatitis in the present series in patients who had had blood counts, venepunctures, or parenteral injections other than blood was no higher than in patients who had not received injections.

No estimate of the frequency of homologous serum jaundice was possible from the data available. However, in previously reported studies the proportion of patients transfused who subsequently developed hepatitis was high. For example, one in every 222 transfusions of blood or plasma at the Peter Bent Brigham Hospital was followed by hepatitis (15) and one in every 21 patients transfused with pooled plasma in Upper New York State developed hepatitis (17). The data suggest that homologous serum jaundice is a problem which is serious enough so that the indications for blood or plasma transfusions should be weighed carefully against the risks involved. Furthermore, the use of pooled plasma or multiple transfusions should be avoided wherever possible since the risk of transmitting homologous serum jaundice is proportional to the number of blood donors used.

It has been demonstrated repeatedly that acute hepatitis can be transmitted by minute quantities of infectious materials. A number of epidemics have been described in which hepatitis apparently resulted from the use of inadequately sterilized needles or syringes (7–13). In the present study, approximately 20 per cent of cases of hepatitis, not following blood or plasma transfusions or injections of arsenical compounds, occurred in patients who had previously had a parenteral inoculation or test. In a control group of patients, the incidence of parenteral inoculation was only 8 per cent. The evidence indicates

that injections of any variety or the withdrawal of blood should be made only with needles, syringes, or lancets which have been properly sterilized. The only practical agent known which will destroy the virus of hepatitis is heat (8). The inadvertent inoculation of patients with the virus of hepatitis may be avoided by boiling or autoclaving all needles, syringes, and lancets before their use in each patient. Sterilization with such agents as phenol (18), ether (18), and merthiolate (19) has been shown to be ineffective. It would not be sufficient to boil or autoclave the needles and syringes used on patients with hepatitis alone. There is considerable evidence either that there are carriers of the virus, or that virus is present in the blood both during the incubation period (20–22) and subsequent to the disease (23).

No satisfactory evidence has been reported as to whether infectious hepatitis and homologous serum jaundice are separate entities or variations of the same disease which depend on the route of infection (24) It is of interest that in this study the clinical, laboratory, and pathologic features of the patients with homologous serum jaundice did not differ from those of patients with acute hepatitis from other causes. The greater severity reported in post-transfusion hepatitis could well be explained by the dosage of the inoculum of virus.

The data presented indicate that acute hepatitis during pregnancy is frequently attended by abortion or premature delivery.

SUMMARY

(1) Approximately one out of every 7 patients hospitalized with acute hepatitis during a 12 year period had been transfused with homologous blood or a blood fraction 5 weeks to 5 months before the onset of the hepatitis. The case fatality rate in 40 patients with homologous serum jaundice was 27.5 per cent.

(2) No clinical, laboratory, or pathologic features other than differences in severity were observed which distinguished between homologous serum jaundice and other varieties of acute infectious hepatitis. Acute infectious hepatitis which occurred during pregnancy was frequently associated with abortion or premature delivery.

(3) Twenty per cent of 183 patients with hepatitis had had a parenteral test or injection of material other than blood or arsenic within 5 weeks to 5 months before the onset of their illness. Only 8 per cent of

258 control patients without hepatitis had had a similar test or injection. The probability that this difference could have occurred by chance is less than one in 100. The data emphasize the hazard of transmitting hepatitis by any procedure in which the skin is pierced, unless adequate sterilization of instruments has been achieved. All syringes, needles, and lancets, whether used for withdrawal of blood or injection of any material, should be boiled or autoclaved before their use in each patient in order to prevent the transmission of hepatitis.

BIBLIOGRAPHY

1. Findlay, G. M., and MacCallum, F. O., Note on Acute Hepatitis and Yellow Fever Immunization, Trans. Royal Soc. Trop. Med. and Hyg., **31:** 297–308 (Nov. 1937).
2. Oliphant, J. W., Jaundice Following Administration of Human Serum, Harvey Lectures, **39:** 254–272 (1944).
3. Neefe, J. R., Stokes, J. J., Jr., Reinhold, J. G., and Lukens, F. D. W., Hepatitis Due to the Injection of Homologous Blood Products in Human Volunteers, J. Clin. Invest., **23:** 836–855 (Sept. 1944).
4. Paul, J. R., Havens, W. P., Jr., Sabin, A. B., and Philip, C. B., Transmission Experiments in Serum Jaundice and Infectious Hepatitis, J. A. M. A., **128:** 911–915, (July 28, 1945).
5. Hanger, F. M., Jr., and Gutman, A. B., Obstructive Type of Jaundice Caused by Arsphenamine, Trans. Assn. Am. Phys., **55:** 179–182 (1940).
6. Sawyer, W. A., Meyer, K. F., Eaton, M. D., Bauer, J. H., Putnam, P., and Schwentker, F. F., Jaundice in Army Personnel in the Western Region of the United States and Its Relation to Vaccination Against Yellow Fever (Part I), Am. J. Hyg., **39:** 337–430 (May 1944).
7. Salaman, M. H., King, A., Williams, D. I., and Nicol, C. S., Prevention of Jaundice Resulting from Antisyphilitic Treatment, Lancet, **2:** 7–8, (July 1, 1944).
8. Sheehan, H. L., Epidemiology of Infective Hepatitis, Lancet, **2:** 8–11, (July 1, 1944).
9. Hartfall, S. J., Jaundice in Rheumatoid Arthritis, Lancet, **2:** 358, (Sept. 9, 1944).
10. Droller, H., An Outbreak of Hepatitis in a Diabetic Clinic, Brit. Med. J., **1:** 623–625, (1945).
11. Marshall, J., Post-arsphenamine jaundice, Proc. Royal Soc. Med., **37:** 453–456, (June, 1944).
12. Darmady, E. M., and Hardwick, C., Syringe-transmitted hepatitis, Lancet, **2:** 106, (July 28, 1945).
13. Hughes, R. R., Post-Penicillin Jaundice, Brit. Med. J., **2:** 685–688, (Nov. 9, 1946).

14. Ravitch, M. M., Personal communication.
15. Sheinberg, I. H., Kinney, T. D., and Janeway, C. A., Homologous Serum Jaundice: A Problem in the Operation of Blood Banks, J. A. M. A., **134:** 841–848, (July 5, 1947).
16. Snell, A. M., Wood, D. A., and Meienberg, L. J., Infectious Hepatitis with Especial Reference to its Occurrence in Wounded Men, Gastroenterology, **5:** 241, (Oct. 1945).
17. Brightman, I. J., and Korns, R. F., Homologous Serum Jaundice in Recipients of Pooled Plasma, J. A. M. A., **135:** 268–272 (Oct. 4, 1947).
18. MacCallum, F. O., Homologous Serum Hepatitis, Proc. Royal Soc. Med., **39:** 655–657. (March 22, 1946).
19. Beeson, P. B., Chesney, G., and McFarlan, A. M., Hepatitis Following Injection of Mumps Convalescent Plasma, Lancet, **1:** 814, (1944).
20. Oliphant, J. W., Gilliam, A. G., and Larson, C. D., Jaundice Following Administration of Human Serum, Public Health Rep., **58:** 1233–1242, (1942).
21. Havens, W. P., Jr., Period of Infectivity of Patients with Experimentally Induced Infectious Hepatitis, J. Exp. Med., **84:** 251–258, (March 1946).
22. Havens, W. P. Jr., Period of Infectivity of Patients with Homologous Serum Jaundice and Routes of Infection in the Disease, J. Exp. Med., **83:** 441–447, (June, 1946).
23. Sawyer, W. A., Meyer, K. F., Eaton, M. D., Bauer, J. H., Putnam, P., and Schwentker, F. F., Jaundice in Army Personnel in the Western Region of the United States and its Relation to Vaccination Against Yellow Fever (Parts II, III, and IV). Am. J. Hyg., **40:** 35–107 (July, 1944).
24. Mirick, G. S., Hepatitis, Modern Concepts, Southern Med. J., **41:** 743–746, (August, 1948).

COLONMETROGRAPHIC STUDIES OF THE EFFECTS OF SECTION OF THE PARASYMPATHETIC NERVES OF THE COLON*

H. WILLIAM SCOTT, JR., AND JAMES R. CANTRELL

Baltimore, Maryland

Received for publication July 11, 1949

The work of Lium (1, 2, 3, 4) and his associates on the etiology of ulcerative colitis has directed interest toward the neurogenic aspects of this disease and the rôle played therein by spasm of the colonic musculature. Recently Dennis (5) has used vagus section in treatment of patients with ulcerative colitis in an effort to reduce spasm and hypermotility of the colon and to slow the intestinal transit time.

The present investigation has been carried out in order to assess the effects of section of the various motor nerves of the colon on the tone and reactivity of the normal large intestine. For this purpose experimental observations have been made in dogs, and clinical studies have been carried out in patients undergoing vagotomy for peptic ulcer.

THE COLONMETROGRAM

The colonmetrogram was described by White and his associates (6) in 1940 as a means of recording manometrically the tone, reflex irritability and sensitivity of the colon as a whole. The apparatus used consists of a vertical glass tube manometer connected on one side to an intravenous drip set and on the other to a 100 cc. Foley catheter (Figure 1). Water, saline or dilute barium sulfate solution may be used as the infusing fluid. The reservoir is filled with fluid at body temperature and the tubing cleared of air. The base line of the manometer is adjusted so that the meniscus of the fluid column is at the zero mark when the tip of the rectal catheter is level with the subject's anus. After inserting the tip of the catheter into the rectum the infusing fluid is permitted to run into the colon at a constant rate of 100 cc. per minute in observations on patients and 25 cc. per minute in studies

* From the Surgical Hunterian Laboratory of the Johns Hopkins University School of Medicine.

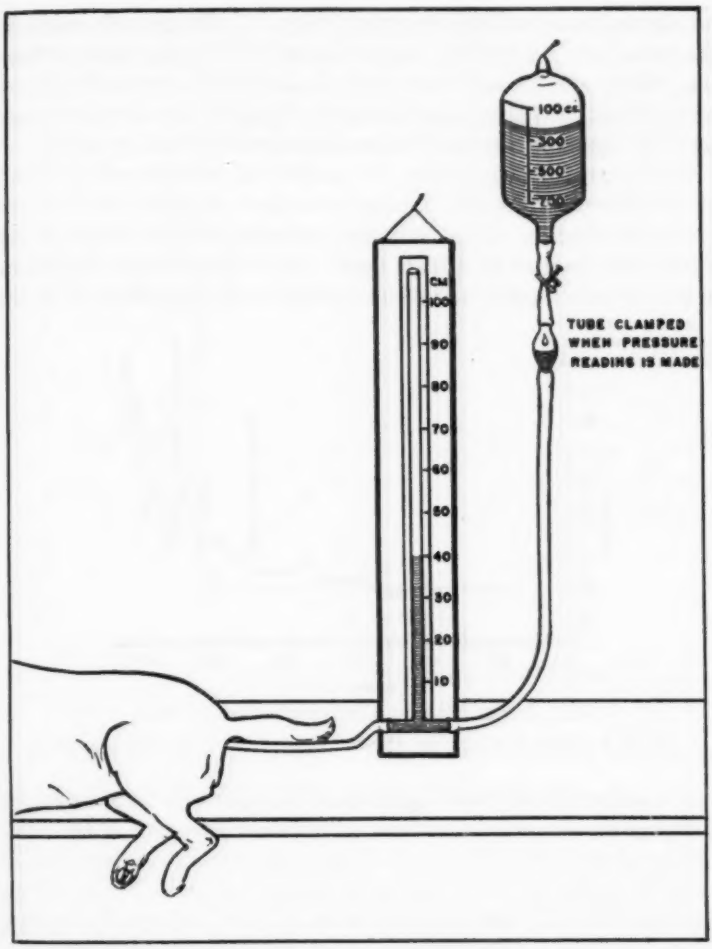


FIG. 1. APPARATUS FOR COLONMETROGRAPHIC STUDIES

on the dog's colon. The pressure recordings are then plotted against the volume of fluid introduced in graphic form.

The normal colonmetrogram in man resembles the normal cystometrogram (6) except for the fact that a four-fold larger volume of fluid is

usually required for filling the colon (Figure 2). The smooth muscle in the colon, as in the bladder, reacts to stretch stimuli by reflex contraction. White and his associates have demonstrated that various types of neurologic lesions are associated with changes in the colonmetrogram (as in the cystometrogram) characteristic of the level of the lesion.

Colonmetry in dogs cannot be carried out satisfactorily without anesthesia—otherwise the intense voluntary straining renders the curves meaningless. An intratracheal catheter prevents closure of the glottis and obviates to a large degree the diaphragmatic component of the defecation pattern. Sodium pentobarbital has proven to be the

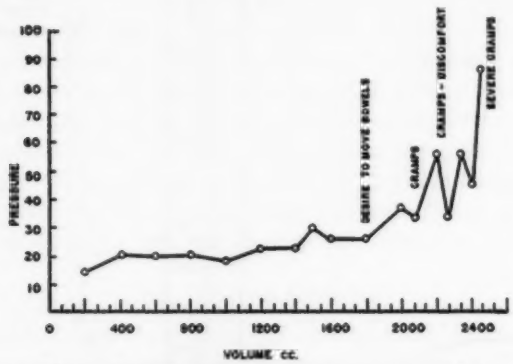


FIG. 2. THE NORMAL COLONMETROGRAM IN MAN
From a patient in this study. Pressure in centimeters of 0.85% saline.

most satisfactory anesthetic agent from the point of view of repetition of dosage for comparative studies and minimal alteration of the reflex activity of the bowel. The use of drugs such as morphine, atropine, or curare is contraindicated because of profound disturbances in colonic responses following their administration. A Foley catheter is necessary to prevent leakage from the anus in dog experiments. Adequate preparation of the bowel for colonmetry in the dog includes a 36 to 48 hour period of starvation and repeated cleansing enemas of tap water 12 hours before the observations are made. Cathartics are to be avoided.

The colonmetrogram in the normal anesthetized dog resembles

closely the normal filling curve of the colon in man except for the smaller volume required to fill the dog's colon (Figure 3). A standard dilute solution of barium sulfate (75 gms. in 1 liter of water plus 5 gms. acacia) used as the infusing fluid in the dog experiments permits the colonmetrogram to be carried out under fluoroscopic control. Opening

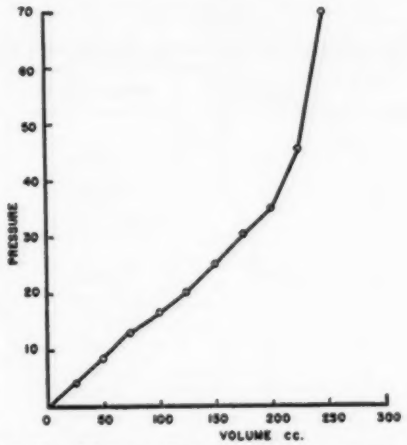


FIG. 3. THE NORMAL COLONMETROGRAM IN THE DOG

Pressure in centimeters of standard dilute barium solution (75 gms. $BaSO_4$ + 5 gms. acacia in 1 liter water).

of the ileocecal valve with reflux of barium into the ileum is taken as the end-point of the filling curve in the anesthetized dog.

METHODS

Adult mongrel dogs weighing 8 to 15 kg. were used in the animal experiments. All animals were anesthetized with sodium pentobarbital given intravenously in doses of 30 milligrams per kilogram of body weight for each experiment. Control colonmetrograms and roentgenograms of the barium-filled colon were made in each instance. After obtaining satisfactory control observations each animal was given a rest period of several days and then subjected to operative interruption of one set of the various motor nerves of the colon. All operations were carried out with aseptic technic; intratracheal ether

or intravenous sodium pentobarbital (30 mg./kilo of body weight) were used for anesthesia. After recovery from laparotomy or thoracotomy, colonmetrographic and roentgenographic observations were made of the colon of each animal, usually beginning at 14 days after operation and thereafter at intervals of 1 month.

TABLE 1

Summary of Colonmetrographic Observations after Section of Nerves to Colon

DOGS	NO. OBSERVATION	PERIOD OF STUDY	MEAN RESULT
Vagus Nerves			
1. C2	4	4 mos.	No change
2. C4	2	1 mo.	No change
3. C7	2	1 mo.	No change
4. C12	4	4 mos.	Slight flattening with return
5. C18	4	3 mos.	No change
6. C28	3	2 mos.	No change
Pelvic Nerves			
1. C24	3	5 mos.	Flat curve
2. C29	3	2 mos.	Flat curve
3. C36	3	3 mos.	Flat curve
4. C37	5	9 mos.	Flat curve
5. C34	2	1 mo.	Flat curve
6. C2	4	9 mos.	Flat curve
7. C38	3	2 mos.	Flat curve
Hypogastric Nerves			
1. C6	6	5 mos.	No change
2. C7	4	3 mos.	Slight flattening with return
3. C9	5	5 mos.	No change
4. C18	4	6 mos.	No change

In the colonmetrograms on patients the procedure described by White was followed. No drugs were used before or during the colonmetrogram.

RESULTS

1. *Vagus nerves.*

Six dogs were used in these experiments. In each instance the vagus trunks were divided just above the diaphragm with excision of 4 cm. segments from each trunk and careful extirpation of the periesophageal

vagal plexus. Colonmetrograms and barium enemas were carried out in these animals following vagotomy for survival periods ranging from 1 to 4 months. A total of 19 colonmetrograms were done during the period of study (Table 1). In 5 animals there was essentially no change

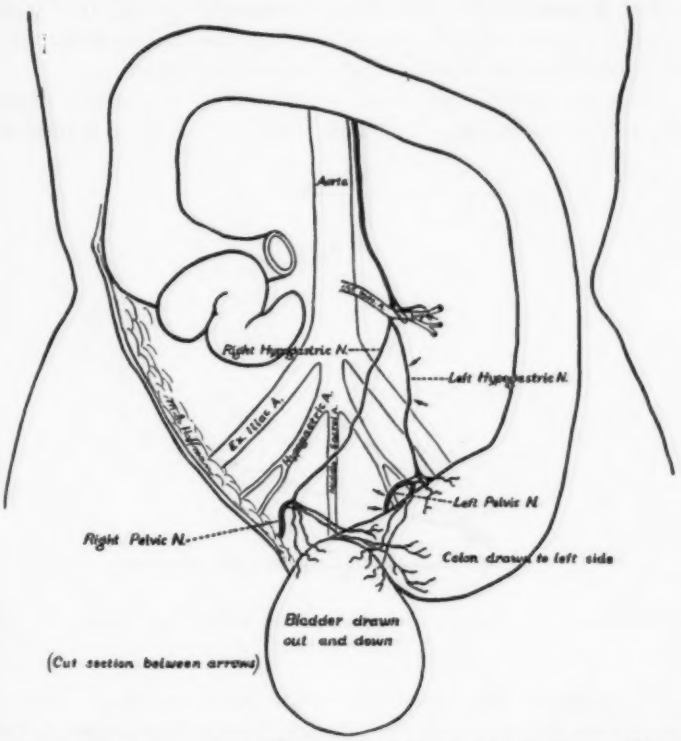


FIG. 4. SKETCH DEPICTING THE HYPOGASTRIC NERVES AND THE PELVIC NERVES IN THE DOG

in the colonmetrograms following vagotomy. In the remaining animal the curve obtained 2 months after operation showed some reduction in tone but subsequent curves were identical with the control colonmetrogram. Roentgenograms of the barium filled colon showed no apparent change in caliber of the bowel after vagotomy in any of the animals. Defecation was normal in these dogs, and no diarrhea was encountered.

2. *Pelvic nerves* (*Nervi erigentes*).

Seven dogs were used in these experiments. Bilateral section of the pelvic nerve trunks was carried out in each animal as shown in Figure 4. Bladder paralysis developed promptly in all animals necessitating repeated catheterization during the post-operative period. In 3 animals a large suprapubic cannula was sutured into the bladder at the time of pelvic nerve division in order to obviate urinary retention.

Colonmetrograms and barium enemas were carried out in these animals for survival periods ranging from 1 to 9 months. A total of 23

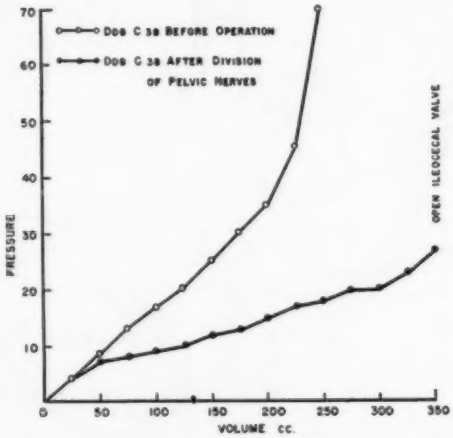


Fig. 5. Typical Colonmetrogram Before and After Pelvic Nerve Division in the Dog

colonmetrograms were done during the period of study (Table 1). Flat curves were obtained consistently in all dogs after pelvic nerve division (Figure 5). Barium enemas showed slight dilatation of the distal half of the colon in most animals, but in no instance did the dilatation reach a degree which suggested megacolon. These dogs defecated in apparently normal fashion. No fecal impaction or diarrhea was encountered.

3. *Hypogastric nerves.*

Four dogs were subjected to division of the hypogastric (sympathetic) trunks in the pelvis as control animals. Four centimeter segments

were excised from the hypogastric trunk on either side as in Figure 4, and damage to the nervus erigens was avoided.

Colonmetrograms and barium enemas were carried out in these animals for periods ranging from 3 to 6 months. A total of 19 colonmetrograms were done. In 3 animals no change was observed in the filling curve of the colon. One dog showed hypotonic curves for 2 months after operation, but subsequent curves during the next 3 months were identical with the preoperative colonmetrogram. Barium enemas showed no change in the appearance of the colon in any of these animals. None of the animals developed any disturbance of bladder function or of defecation. No fecal impaction or diarrhea was encountered.

4. *Observations in Patients* (*Vagus nerves*).

In 10 patients undergoing vagus nerve section for peptic ulcer colonmetrograms and barium enemas were carried out before operation and two weeks after vagotomy. In no instance did the colonic filling curve following vagotomy differ from the preoperative colonmetrogram. No changes were observed in the roentgenograms of the barium filled colon of any patient after vagotomy.

COMMENT

After division of the nervi erigentes in the dog colonmetrograms show consistently hypotonic curves. These flat curves indicate that section of the pelvic nerves results in reduction in the tone and reactivity to stretch stimuli of the colon of the anesthetized dog. These changes were not observed after division of the vagus nerves or the hypogastric nerves in dogs. No changes were observed in the colonmetrograms of clinical patients after vagotomy.

These observations are in accord with the experimental evidence as to the function of the motor nerves of the colon accumulated by Ivy and his associates (7) by electrical stimulation. Ivy found that electrical stimulation of the distal end of the divided pelvic nerves in dogs, monkeys, and pigs causes contraction of both longitudinal and circular muscular coats of the descending and distal colon. Stimulation of the vagus nerves, both in the neck and above the diaphragm was uniformly ineffective in producing a response of the colon in the dog, and only weak inconstant contractions of the cecum were occasionally observed in pigs and monkeys. Stimulation of the distal end of the

hypogastric nerves produced an inconstant circular contraction of the musculature of the distal colon in about half the animals studied.

The results of pelvic nerve division in this group of dogs are also in agreement with the colonmetrographic observations made by White and his associates (6) on patients with destructive lesions involving the pelvic parasympathetic nerves, the cauda equina, and the sacral cord. In such patients the curves were found to be consistently hypotonic in type. Conversely, in patients with lesions of the motor fibers in the brain or in the descending spinal tracts the colonic filing curves were hypertonic in type. White concluded that the peristaltic contractions of the colon are a form of "stretch-reflex" with afferent and efferent arcs mediated through the pelvic parasympathetic nerves to the sacral segments of the cord.

The steep rise in intracolonic pressure and the powerful peristaltic contractions which occur as the normal colon is filled to capacity are not observed after division of the nervi erigentes. The most likely explanation for these differences in colonic activity is that expulsive contraction of the distal colon is abolished or greatly reduced by interruption of the pelvic parasympathetic reflex arc.

Although slight dilatation of the distal colon was observed in dogs after pelvic nerve division, nothing suggesting megacolon was encountered. No colonic dilatation was observed after hypogastric nerve section or vagotomy.

On the basis of these experimental observations it would seem reasonable to conclude that surgical attempts to alleviate spasm and hypermotility of the colon by interruption of its motor nerves had best be directed toward section of the pelvic parasympathetic nerves. If it should prove to be anatomically feasible in man to divide the pelvic parasympathetic fibers to the colon without interrupting the bladder fibers an operative procedure to reduce the tone and reactivity of the distal colon might be developed which would be of benefit in certain instances of "spastic colitis" and possibly in early ulcerative colitis.

REFERENCES

1. Lium, R. and Porter, J. Observations on the Etiology of Ulcerative Colitis. I. Preparation, Care and Secretions of Colonic Explants in Dogs, Arch. Int. Med., **63:** 201, 1939.

2. Lium, R. Observations on the Etiology of Ulcerative Colitis. II. Effect of Induced Muscle Spasm on Colonic Explants in Dogs with Comment on Relation of Muscular Spasm to Ulcerative Colitis, Arch. Int. Med., **63:** 210, 1939.
3. Lium, R. and Porter, J. E. Observations on the Etiology of Ulcerative Colitis. III. The Distribution of Lesions and Its Possible Significance, Am. J. Path. **15:** 73, 1939.
4. Lium, R. Observations on the Etiology of Ulcerative Colitis. IV. The Rectometrogram and the Rectal Reactions of 8 Normal Subjects and One Patient with Ulcerative Colitis—Before and After Spinal Anesthesia, Am. J. Med. Sci., **197:** 841, 1939.
5. Dennis, C., Eddy, F. D., Frykman, H. H., McCarthy, A. M. and Westover, D. The Response to Vagotomy in Idiopathic Ulcerative Colitis and Regional Enteritis, Ann. Surg., **128:** 479, 1948.
6. White, J. C., Verlot, M. G., and Ehrentheil, O. Neurogenic Disturbances of the Colon and Their Investigation by the Colonmetrogram, Ann. Surg., **112:** 1042, 1940.
7. Wells, J. A., Mercer, T. H., Gray, J. S. and Ivy, A. C. The Motor Innervation of the Colon, Am. J. Physiol., **138:** 83, 1942.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Bulletin)

A Primer of Electrocardiography. By BURCH AND WINSOR. Second Edition. 245 pp. $4.50. *Lea and Febiger, 1949.*

This book presents in logical order the fundamentals of electrical physiology which are necessary for the proper understanding of clinical electrocardiography. It is a book to be contrasted with the usual type of electrocardiographic manual which merely presents a series of clinical examples with brief explanations and interpretations. This primer is not just a collection of electrocardiographic patterns, but is a beautifully organized and clearly illustrated logical approach to the method and the meaning of the results in physiological terms. It is a superb text for students in a course of electrocardiography or for a practicing physician who wishes to understand something more than the usual empirical patterns obtained in cardiac disease.

It is obviously the result of much experience in the successful teaching and study of electrocardiography by these authors.

E. V. N.

Clinical Auscultation of the Heart. By LEVINE AND HARVEY. 327 pp. $6.50. *W. B. Saunders Company.*

This book follows as a natural sequence the already well known publication on clinical heart disease by the senior author of this present book. "Clinical Auscultation of the Heart" has been written to emphasize the importance of adequate physical examination in cardiological diagnosis, in which the value of auscultation and the information to be obtained by the use of the stethoscope is stressed. Chapters deal with the variations to be found in normal heart sounds, auscultatory findings in cardiac irregularities and the significance of cardiac murmurs. The text is amply illustrated with phonocardiograms recorded with simultaneous electrocardiograms and the book makes enjoyable reading. It is unnecessary to emphasize the fundamental clinical importance of much that is written, representing as it does the teaching and experience of one of this country's eminent clinical cardiologists. The therapeutic references in the book will be of considerable help to the practicing internist and the clinical approach to auscultatory problems at the bedside should be of help to future generations of students.

Realizing that this book has been written as a practical guide to clinical auscultation it seems a pity that the authors did not enlarge the scope of the book as the phonocardiographic tracings have been used mainly to illustrate and to help visualize points taken up in the discussion in the text. Phonocardiography has contributed much to a better understanding of the mechanism of heart sound production and with the large number of tracings included, greater use could have been made of them. In many instances it is impossible to determine accurately the duration and type of component vibration which has been recorded.

It would have been of great value if the authors had extended their discussion to a more complete analysis of the mechanism of heart sound recordings and also to debate

more fully some of the controversial aspects of heart sound production. However, the important emphasis made in this book is that often an all-important and life-saving diagnosis can be made by careful auscultation as long as the attending physician is aware of all the information which can be derived from such a relatively simple procedure. This book goes a long way in condensing this information and presenting it in an easily accessible form. As such it is recommended to all who confront this problem and to students who will always worry about the opening snap of the mitral valve.

B. C. S-S.

Electrocardiography and Clinical Disorders of the Heart Beat. Sir THOMAS LEWIS. 285 pp. 25/. *Shaw and Sons Ltd., London.*

One need not comment at any length on this edition of these very popular sound treatises which should be read and re-read by every student in medicine, whether he is in medical school or is a graduate who intends to continue to examine patients. It is fortunate that two works of this great physician have been combined in one compact, nicely printed volume.

E. V. N.

Handbook of Materia Medica, Toxicology, and Pharmacology. 4th Edition. By FORREST R. DAVISON. 730 pp. $8.50. *The C. V. Mosby Co., St. Louis,* 1949.

The fourth edition of Davison's Handbook is divided into two parts. The first part considers principles of pharmacology, materia medica, prescription writing and toxicology, while the second contains a discussion of the individual drugs. The print is quite small. In general the book is an example of the categorical approach, which would make a distinct discipline of therapeutics, with little integration with current concepts of disease mechanisms. In addition, there are occasional major errors such as the confusion of rheumatic heart disease with bacterial endocarditis. Finally, there is a neglect of certain topics, as for example the discussion of electrolytes which is limited to six lines. For the above reasons the book cannot be recommended.

C. G. Z.

Measurements of Public Health. By F. A. E. CREW. pp. xiii + 243. 18 shillings. *Oliver and Boyd, Edinburgh,* 1948.

The author, who has had a distinguished career in the field of animal genetics, has more recently undertaken the professorship of public health and social medicine in the University of Edinburgh. The book is more specialized in scope than the title would suggest. It consists of a series of essays based on the data in the Registrar-General's 1945 report for Scotland and is essentially a discussion of the state of the public health in Scotland, written in non-technical language for interested laymen as well as medical men.

The principal topics dealt with are the population structure by age and sex, birth- and fertility-rates, illegitimacy-rates, marriage- and divorce-rates, age at marriage, and death-rates by principal causes of death, including stillbirths and infant and maternal mortality. The book also includes topics such as multiple-births, sex-determination and mutation, which appear rather remote from its main purpose, but serve as an indication that the author has not entirely deserted his old love. Unfortunately these sections seem to the reviewer to be the weakest from an expository point of view, since free use is made of the current terminology in genetics, not always with a clarifying explanation.

It may not be too much of a distortion to regard Professor Crew as a relatively "pure" scientist who has entered a career where almost any recommendation that is made involves intricate sociological, economic, political, and psychological questions. In this situation, one possible attitude for the pure scientist has been described by another outstanding geneticist, R. A. Fisher:

> "The investigator undoubtedly best preserves both his scientific reputation and his peace of mind, who refrains from any opinion respecting human affairs, and declines the responsibility for any practical action which might be based upon the facts he has brought to light. If such a one were to admit that mankind might, with advantage, make more use of scientific knowledge of all kinds, his practical policy would, apparently, go no further than to advocate a more general diffusion of this scientific knowledge, its application to mundane affairs being relegated to others (unspecified) who might make it their particular business."

Professor Crew does not adopt this attitude, but he has not lost his scientific detachment and is in no hurry to rush in with a host of proposals for an immediate cure of Scotland's ills. Perhaps he might have ventured more than he has, for most of his definite recommendations seem rather obvious, and on some important and difficult issues it is not clear what he would propose.

As is well-known, Scotland is likely to face the problems of an aging population, with a net reproduction rate fluctuating around unity and a drain by emigration on some of her best reproductive material. Unsatisfactory aspects of her public health are the relatively high infant and maternal mortality rates and the high illegitimacy rate (1 in 12 births). Professor Crew makes the point that if Scotland wishes to multiply this must be done now, since in the future the problem of caring for the aged will be a sufficiently great burden to deter her from assuming the additional load caused by an increase in children. With regard to emigration, she can afford to export her surplus women of marriageable age, but not her young married couples. He quotes investigations on the causes of infant and maternal mortality which indicate that in both cases a substantial reduction in death rates can be expected from increased availability of good medical care. He makes a moving plea for measures that will remove the stigma of illegitimacy from the child and ensure as good natal care for the unmarried as for the married mother.

William G. Cochran

Pathology of Tumors. By R. A. Willis, D. Sc., M.D., F.R.C.P. 992 pp., 500 illustrations. $10.00. *Butterworth and Co., Ltd., London, and The C. V. Mosby Co., St. Louis.* 1948.

This is an excellent and very useful book on the pathology of tumors,—indeed, in the opinion of the reviewer, it is one of the finest in the field, in any language. It is a book that has grown out of a broad experience with tumors and a genuine devotion to the subject.

Those who may be so inclined may cavil at the fact that not all tumors about which they may seek information are described in the book; but any such objection cannot detract from the value of this comprehensive, well-illustrated work. Because of the enormous variety of tumors, no single author has ever adequately described them all. Indeed, it is hardly possible that that could be accomplished in any treatise short of an encyclopedic compilation prepared by a great many different students of tumors; and even then many of the types and variations of tumors would, with little doubt, be inadequately treated.

Professor Willis never hesitates to express his own opinion plainly about matters concerning which opinions may differ. He does this, however, not in a dogmatic manner, but

with a clear, concise statement of the reasons for his preference, and he reserves the right to alter his opinion if future experience warrants doing so.

The book is a refreshing one, for it was obviously written with pleasure, and it therefore represents one of the all too rare modern medical books in which the personality of the author lives in the pages, and which one can read with pleasure as well as with profit.

A. R. R.

Problems of Early Infancy—Transactions of the Second Conference, March 1948. Edited by MILTON J. E. SENN, M. D. Price $1.00. Pages 120. *Publication of The Josiah Macy Jr. Foundation, New York, N. Y.*

The first part of this volume contains twelve reports whose wide range of subject matter is exceeded only by that of the discussions following each group of talks. The first eight of these reports deal largely with the psychological problems of pregnancy, their possible influence on fertility, the cause and complications of pregnancy, the development of the fetus and the delivery of the patient. The use of relaxation methods in obstetrics practice is discussed by Dr. Albert Vollmer, and emotion in pregnancy and labor as related to childbirth by S. W. Goodrich, M.D. Other presentations include the myelinization of the central nervous system in relation to function by Margaret A. Kennard. This subject is still but poorly understood. However, there is strong evidence that myelinization, as it results from use, helps to bring about increasing efficiency of function. A memorandum on emotional disorganization in this culture by James Clark Moloney, M.D. offers a plea for affectionate understanding of the emotional needs of infants and children as a guide toward their education, rather than training. He points out that many mothers because of their own narcissistic needs and neurotic tendencies are incapable of mother love and that they are unconsciously aided and abetted by many physicians and nurses whose authoritative and sometimes irrational behaviour place a cold screen of mechanical devices between mother and child. The last presentation in this group by Dr. Preston A. McLendon is entitled "Modern Nurseries—New Design". It contains a description of the nurseries of the George Washington University Hospital. This is a timely paper; obstetricians, pediatricians and Public Health authorities are becoming more and more aware that most hospital nurseries are inadequate to provide either rational mother-child relationship or adequate control of infection. The breaking up of large nursery groups into small units as has been done in the new nurseries offer a practical approach to both of these problems.

This volume contains as a supplement reports of five talks concerned with the impact of World War II upon the emotional development of children in the occupied countries of Europe. Four of these were presented by European pediatricians visiting the International Congress of Pediatrics in New York in 1947. These give first hand information to American pediatricians. The fifth report in this group was made by Dr. David Levy who travelled abroad extensively shortly after the end of the war. This is a very worthwhile little volume presenting a variety of challenging ideas to Americans interested in child development.

J. B. H.

The Premature Infant. JULIUS H. HESS, M.D., EVELYN C. LUNDEEN, R.N. 381 Pages. 101 illustrations. Price $6.00. *J. B. Lipincott Co., Philadelphia,* May 1949.

This is the new, thoroughly up-to-date second edition of the well-known book on premature infant care by Hess and Lundeen. Here is described in detail the care given to pa-

tients in the premature infant stations in the Sarah Morris Hospital, Chicago. The book should be of great value to Public Health nurses, and nursery personnel as nursing technique, procedures and teaching methods, both for hospitals and homes use, are outlined in the greatest detail. The chapters devoted to the medical care of the premature infant are complete only rather superficially but because of the detailed information given about some practical aspects of the problem, for instance, dosage of chemotherapeutic agents, methods of administrating transfusions, etc., they should be of considerable value to the practicing physicians. The chapter discussing the prognosis for premature infants, particularly as regards mental development represents an important contribution to our knowledge in this direction. The final chapter in which city-wide and state-wide plans for the care of premature infants are discussed is of particular interest. The concise description of the plans operating or being developed in eleven states should prove of value to Public Health officers and others interested in the all-important phases of community planning, the only real way in which to reduce mortality rates among premature infants.

J. B. H.

BOOKS RECEIVED

Atlas of Roentgenographic Positions in two volumes. By VINITA MERRILL. 708 pp. $30.00. *C. V. Mosby Co., St. Louis, Missouri.*

Essentials of Orthopaedics. By PHILIP WILES. 486 pp. $10.00. *The Blakiston Co., Philadelphia, Pa.*

Hematology for Students and Practitioners. Revised 2nd Edition, By WILLIS M. FOWLER. 535 pp. $8.50. *Paul B. Hoeber, Inc., New York.*

The Neurosis of Man. By TRIGANT BURROW. 428 pp. $7.50. *Harcourt, Brace and Co., New York.*

The Practice of Refraction. By Sir STEWART DUKE-ELDER, 5th Edition. 317 pp. $6.25. *C. V. Mosby Co., St. Louis, Missouri.*

The Science and Art of Joint Manipulation. 2nd Edition. By JAMES MENNELL. 215 pp. *The Blakiston Co., Philadelphia, Pa.*